DEVELOPMENT AND CHARACTERIZATION OF FUROSEMIDE LOADED NANOPARTICLES

Dissertation Submitted in partial fulfilment of the requirement for the

Award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

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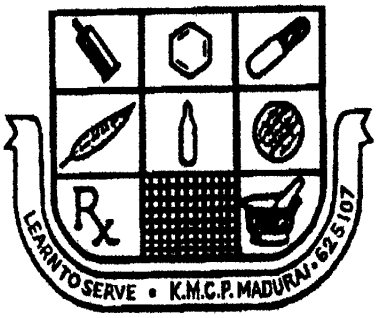

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APRIL-2014

CERTIFICATE

This is certify that the dissertation entitled "**DEVELOPMENT AND CHARACTERIZATION OF FUROSEMIDE LOADED NANOPARTICLES"** submitted by **Mr. J.MOHAMED MEERAN**,(**Reg.No.261210105**) in partial fulfilment for the award of Master of Pharmacy in Pharmaceutics under the Tamilnadu Dr.M.G.R Medical University, Chennai, done at **K.M.COLLEGE OF PHARMACY**, Madurai-625107, is a bonafide work carried out by his under my guidance and supervision during the academic year **APRIL-2014.** The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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CONTENTS

1. INTRODUCTION

Ideal drug delivery systems deliver the drug at a rate dictated by the need of the body over the period of treatment and it channels the activity entirely solely to the site of action. At present no available drug delivery systems can achieve all these goals[1].

1.1. TARGETTED DRUG DELIVERY SYSTEM

The nanotechnology carrier main advantage for site specific or organ specific particulate drug carrier include microparticulate, nanocarriers, lipid based carriers and colloidal carriers this drug carrier needs some special character and limitation.

1.2. LEVELS OF DRUG TARGETING

The drug delivery in colloidal carriers paves way for targeting drugs to specific sites. The various mechanism involved in drug reach to specific site as follows[2],

a) Passive target
b) Active target
   i) First order targeting
   ii) Second order targeting
   iii) Third order targeting
c) Legend mediated targeting
d) Physical targeting
e) Dual targeting
f) Double targeting
g) Combination targeting

a) PASSIVE TARGETING

In passive targeting the particles system is captured by physiological uptake mechanism such as filtration or macrophage (RES) sequestration. Then the passive involved for drug concentration in plasma and blood levels. The passive targeting is concentration dependent. So, they do not need external energy.

b) ACTIVE TARGETING

The active targeting is attachment of a main moiety in particles surface. Such as monoclonal antibodies or carbohydrate like glucose, galactose, glucose-6-phosphate. The role of an ideal carrier is to transport the required amount of drug to a highly specific site. Which may be organ, tissue or organneller related, the carrier also having property of accumulate at specific receptors and specific organeller with the cells.

i) FIRST ORDER TARGETING

The first order targeting means delivery of drug to a particular organ. The first order targeting more effective on selection of carrier.

ii) SECOND ORDER TARGETING

The second order targeting to a specific cell type. This method widely used for the particular therapeutic purpose.

iii) THIRD ORDER TARGETING

Third order targeting based on a structure within a cell. The third order targeting is essential for gene delivery and targeting of an exogenous DNA to the nucleolus. It is prerequisite for gene expression. The active targeting more specific for kupffer cells of the liver and parenchymal cells like hepatocytes.

c) LEGEND MEDIATED TARGETING

In this method the drug molecules incorporated with lipo-proteins. The lipo-proteins from natural or synthetic origin and they have low density. The carrier molecules having lipo-protein nature. So, they easily modified their structure and accumulate the specific target like origin, tissue and organelles site.

d)PHYSICAL TARGETING

The physical targeting based on external levels (ex-vivo). The physical targeting basic mechanism is drug release from the carrier on external condition or environment EX, The thermo-sensitive nanoparticles may be used for selective release of the content after specific localization like photodynamic therapy EX, doxorubicin nanoparticles.

e) DUAL TARGETING

Dual targeting means, the drug molecules having more specific synergistic force with specific organs or tissues. The nano formulation to extend the half life of the drug molecules and it will enhance the penetration of drug in cell membrane.

f) DOUBLE TARGETING

Double targeting means, the drug molecules reached to specific site by active and passive mechanism. The specific site targeting of drug molecules based on selection of suitable carrier. The combination made with spatial and temporal control of drug delivery.

g) COMBINATION TARGETING

The combination targeting for site specific delivery of proteins and peptides. The targeting systems are equipped with carrier and polymer. This method more specific for the gene therapy.

1.3. NEED OF VESICULAR, COLLAIDAL, MICRO AND NANOCARRIER [3]:

- ✓ Better drug delivery to certain stubborn or impermeable of body.
- ✓ Owing to their small size, chemistry and distribution these carriers have better Bridged gaps between and function of biomolecules.
- ✓ Reaching of micron or nano range with these particles enables them to be a Highly potential carrier in many biological molecules as proteins. e.g., DNA, viruses and xenobiotics.
- ✓ Better targeting to body tissues and sites where action is required, elimination of side effects and adverse effects.
- ✓ Owing to size, nature and chemistry, these systems give better drug permeability from biological membranes and helps in solubilization of some practically insoluble drugs and hence solve bioavailability problems of many drugs.

- ✓ It involves overlap of biotech, and information technology, might result in many important application in life science including area of gene therapy, drug delivery, imaging, biomarkers and novel drug discovery techniques.
- ✓ It also offers an attractive solution for transformation of bio-systems, and provide a broad platform in several areas of bioscience.
- ✓ The surface properties of carrier can be modified for targeted drug delivery e.g. small molecules, proteins, peptides and nucleic acids loaded nanoparticles are not recognized by immune systems and efficiently targeted to particular tissue types.
- ✓ Targeted drug carriers reduce drug toxicity and provide more efficient drug distribution. The drug carrier to enhance half life of the drug molecule and increase in bio-distribution.
- ✓ Drug carriers better penetrate tumors due to their constitution containing pores ranging from 100- 1000 nm in diameter, sometime liphophilic nature carrier used for nanoparticles formulation enhance the penetration nature of drug targeted site.

LIMITATION:

- Drug carriers exhibits difficulty in handling, storage, and administration because of susceptibility to aggregation.
- It has unsuitability for less potent drugs.
- But the key of concern is related to its small size as nanocarriers can gain access to unintended environments with harmful consequences e.g., It can cross the nuclear envelope of a cell and cause unintended genetic damage and mutations.

Table 1. VARIOUS CARRIER BASED DOSAGE FORMS[3]

S.NO	Carrier system	Size range	Feature	Method preparation
1	Nanoparticles	10-1000nm	Submicron- sized colloidal systems, Biodegradable or not	
2	Solid lipid nanoparticle	50-1000nm	Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipids coating.	High-pressure homogenization Microemulsion formation Precipitation As lipid nanopellets
3	Polymeric nanoparticle	10-1000nm	Sub-nanosized colloidal structures composed of synthetic or semi-synthetic polymers	
4	Ceramic nanoparticle	<50nm	Made up of inorganic (ceramic) compounds such as silica, titania and	

			alumina.	
5	Nanotubes and Nanowires		Self-assembling sheet of atoms arranged in the form of tubes and thread-like structures of nanoscale range	Surface functionalization
6	Functionalized Nanocarriers/ Quantam dots		Combination of functionalities of biomolecules and non-biologically derived molecular species	
7	Liposomes	25nm-100µm	Microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartments	Mechanical dispersion Solvent dispersion Detergent removal
8	Lipid emulsion	Lipid globules 1-100nm	Multicomponent fluid made of water,	o/w w/o

			A hydrophobic liquid, One or several surfactants resulting in a stable system	w/o/w w/o/o
9	Lipidmicrotubules / microcylinders	<1µm	Self organizing system in which surfactants crystallize into tightly packed bi layers that spontaneously form	Self emulsification
10	Lipid microbubbles	Few microns	Gas filled microspheres stabilised by phospholipids.	Sonication
11	Lipospheres	0.2-100µm	Water dispersible solidmicro particles composed of solid hydrophobic fat core stabilized by a monolayer of phospholipids molecules embedded in a microparticle surface	Melt method Multiple microemulsion Cosolvent method Preincorporation into lipophilic carrier
12	Ethosomes	-	Noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation	

13	Multicomposite ultrathin capsules	50nm to few micron	Molecular assemblies of tailored architecture having layer-by-layer adsorption of oppositely charged macromolecules onto colloidal particle	Langmuir-Blodgelt technique and chemiosorption from solution
14	Aquasomes	60-300nm	The particle core is composed of noncrystalline calcium phosphate or ceramic diamond and is covered	Self-assemblin hydroxyapatite by co-precipitation method
15	Pharmacosomes		Pure drug vesicles formed by the Amphiphilic drugs	
16	Dendrimers		Macromolecular compounds that consist of a series of branches around an inner core	Polymerization
17	Colloidosomes	-	Solid microcapsules which are hollow, elastic shells	Self-assembly of colloidal particles at the interface of emulsion droplets

18	Niosomes	10 to 1000 nm	Non-ionic surfactant vesicles are bilayered structures	

1.4. PRIMARY GOALS OF NANO BIO – TECHNOLOGIES[3]:

- ✓ More specific drug targeting drug delivery,
- ✓ Reduction in toxicity while maintaining therapeutic effects,
- ✓ Greater safety and biocompatibility,
- ✓ Faster development of new medicine.

1.5. NANOPARTICLES INTRODUCTION

Nanoparticles are the simplest form of structures with sizes in the nm range. In principle any collection of atoms bonded together with a structural radius of < 100 nm can be considered a nanoparticle.

These can include, e.g., fullerens, metal clusters (agglomerates of metal atoms), large molecules, such as proteins, and even hydrogen-bonded assemblies of water molecules, which exist in water at ambient temperatures.

Nanoparticles are very commonplace in nature - for instance proteins exist in almost all biological systems, metal-oxide nanoparticles are easily produced, etc.,

Size Ranges of Particle

- Coarse particles: >10µm
- Fine particles: ~1 µm
- Ultrafine (nano) particles: <0.1 (100nm)

ADVANTAGES OF FEATURE OF NANOPARTICLES[4]

- ❖ Bioavailability
- ❖ Dose proportionality
- ❖ Decreased toxicity

- Smaller dosage form (i.e., smaller tablet)
- Stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticle dosage forms
- Increased active agent surface area result in a faster dissolution of the active agent in an aqueous environment, such as the human body, faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity
- Controlled rate of drug release,
- Greater patient convenience and or better patient compliance,
- Easy handling of nanoparticles prepared in the powder form,

1.6. NANOTECHNOLOGY IN MEDICINE: THERAPEUTIC APPLICATION AND DEVELOPMENTS [5]

The application of nanotechnology to medicine, known as nanomedicine, concerns the use of precisely engineered materials at this length scale to develop novel therapeutic and diagnostic modalities.

The use of materials in nanoscale provides unparallel freedom to modify fundamental properties such as solubility, diffusivity, blood circulation half-life, drug release characteristics, and immunogenicity. In the last two decades, a number of nanoparticles-based therapeutic and diagnostic agents have been developed for treatment of cancer, diabetes, pain, asthma, allergy, infection, and so on. Many advantages of nanoparticle-based drug delivery have been recognized. It improvers the solubility of poorly water-soluble drugs, prolong the half-life of drug of drug systemic circulation by reducing immunogenicity, release drug at a sustained rate or in an environmentally responsive manner and thus lowers the frequency of administration. Deliver drugs in a target manner to minimize systemic side effects and delivers two or more drugs simultaneously for combination therapy to generate a synergic effect and suppress drug resistance.

As a result, a few pioneering nanoparticle-based therapeutic products have been introduced into the pharmaceutical market and numerous ensuing products are currently under clinical testing or are entering the pipeline.

1.7. NANOPARTICLE – BASED THERAPEUTIC APPROVED FOR CLINICAL USE:

In the past two decades, there has been a progressive increase in the number of commercially available nanoparticle-based therapeutic products. A global survey conducted by the European science and technology observation in 2006 showed that more than 150 companies are developing nanoscale therapeutics

So far, 24 nanotechnology-based therapeutic products have been approved for clinical use, with total sales exceeding $5.4 billion. Among these products, liposomal drugs and polymer-drug conjugates are two dominant classes, accounting for more than 80% of the total amount.

Table 2. MARKETED AVAILABLE NANOMEDICINES

Composition	Trade name	Company	Indication	Administration
Liposomal platforms				
Liposomal amphotricin B	Abelcet	Enzon	Fungal infection	i.v
Liposomal IRIV	Epaxal	Berna biotech	Hepatitis B	i.m
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy	Topical
Liposomal morphine	Depodur	Skye pharma	Post surgical analgesic	Epidural
Polymeric platforms				
PEG-anti-VEGF aptamer	Macugen	OSIpharma	Age-related macular degeneration	i.m
PEG-GCSF	Neulasta	Amgen	Neutropenia associated with cancer chemotherapy	s.c
PEG-L-asparaginase	Oncaspar	Enzon	Acute lymphoblastic leukemia	i.v , i.m
Poly(allylamine hydrochloride)	Renagal	Genzyme	End – stage renal disease	Oral
Other platforms				
Albumin –bound paclitaxel	Abraxane	Astrazeneca	Metastatic breast cancer	i.v
Nano- crystalline aprepitant	Emend	Elan	Anti – emetic	Oral

Table 3.ONGOING CLINICAL TRIALS IN NANOMEDICINES

Composition	Trade name	Company	Indication	Administration	Status
Liposomal platforms					

Liposomal annamycin	L- annamycin	Callisto	Acute lympocytic leukemia	i.v	Phase I
Liposomal doxorubicin	Sarcodoxome	Gp – pharm	Soft tissue sarcoma	i.v	Phase I /II
Liposomal lurtotecan	OSI-211	OSI pharma	Ovarian cancer	i.v	Phase II
Liposomal vincristine	Onco TCS	Enzon	Non – Hodgkin lymphoma	i.v	Phase II /III
Polymeric platforms					
PEG-uricase	Puricase	Phoenix	Hyperuricemia	i.v	Phase III
Polycyclodextrin camptothecin	IT – 101	Insert therapeutic	Metastatic solid tumors	i.v	Phase I
Polyglutamate paclitaxel	Xyotax	Cell therapeutic	Ovarian cancer	i.v	Phase III
PEG-nalioxol	NKTR-118	Nectar	Opiod-induced constipation	Oral	Phase I
Other platforms					
Nanoemulsion-based therapy	MB-001	Nanobio	Herpes labialis	Topical	Phase II
Nanoemulsion-based therapy	NB-002	Nanobio	Onchomycosis	Topical	Phase I/II

Table 4. PRECLINICAL NANOMEDICINES

Composition	Therapeutic	Indication
Polymeric micells		
Pluronic block copolymers	Doxorubicin	Various cancers
Polymer-lipid hybrid nanoprticles	Doxorubicin	Solid tumors
Polymersomes	Hemoglobin	Oxygen carrier
Poly(vinyl alcohol) polymeric micells	PVA polymer antitumor activity	Neuroblastoma, melanoma
Dendrimers		
Folic acid –PAMAM dendrimers	Methotrexate	Epithelial cancer
Polypropylenemine		
Albumin-based nanoparticles		
Albumin-bound nanoparticles	doxorubicin , methotrexate	various cancers
Polysaccharide-based nanoparticles		
glycol chitosan nanoparticles	doxorubicin	solid tumors

1.8. NANOMEDICINE MECHANISM [6]

When a nanomaterial enters the human body, it immediately binds to various proteins and amino acids. The molecules with which the particles will attach themselves to determine where it will.

This binding process also affects the behaviour of the particle within the body. Amino acids and proteins that coat nanoparticles change their shape and surface properties, potentially increasing or reducing characteristics such as toxicity, or in medical applications, the ability of particles to deliver drugs to target cells.

To create the new method, the team used a variety of chemicals to probe the surface of various nanoparticles, using techniques already developed by Xia. The size of a nanoparticle and its surface characteristics determine the types of materials with which it will link. Once the size and surface characteristics are known, researchers can create "fingerprints" that identify the ways in which a given particle will interact with biological molecules. These fingerprints allow to predict how the nanoparticles might behave once you're inside the body.

This information will allow us to predict where a particular nanomaterial will, the human body, and whether or not it will be taken by certain cells, this in turn will give us a better idea that nanoparticles may be useful for drug delivery and which can be dangerous to humans or the environment.

1.9. CLASSIFICATION OF NANOPARTICLES [7,8]

A) IN ONE DIMENSION (Thin surface coating)

One-dimensional systems, such as thin films or manufactured surface.

B) IN TWO DIMENSIONS

a) CARBON NANOTUBES

Carbon nanotubes are a new form of carbon molecule. Wound in a network of carbon atoms, these hollow cylinders can have diameters as small as 0.7nm and reach several millimeters in length. Each end can be opened or closed by a fullerene half-molecule. These nanotubes can have a single layer (like a straw) or several layers (like a poster rolled in a tube) of coxial cylinders of increasing.

C) IN THREE DIMENSION

a) FULLERENES (Carbon 60)

Fullerenes are spherical cages containing from 28 to more than 100 carbon atoms (see schematic representation opposite fullerenes are a class of materials displaying physical properties.

They can be subjected to extreme pressures and regain their original shape when the pressure is released. These molecules do not combine with each other, thus giving them major potential for application as lubricants.

b) DENDRIMERS

Dendrimers represent a new class of controlled-structure polymers with nanomeric dimensions. They are considered to be basic elements for large-scale synthesis of organic and inorganic nanostructure with dimension of 1 to 100nm then displaying unique properties .

Compatible with organic structure such as DNA, they can also be fabricated to interact with metallic nano-crystals and nano-tubes or to possess an encapsulation capacity.

c) QUANTUM DOTS

It represents a special form of spherical nanocrystals from 1 to 10nm in diameter. They have been developed in the form of semiconductors, insulators, metals, magnetic materials or metallic oxides.

1.10. PRODUCTION PROCESS OF NANOPARTICLES[7]

The nanoparticles are prepared for following techniques,

1) Dispertion - based process
2) Precipitation – based process
3) Interfacil polymerization
4) Nanoparticles formation by desolvation of macromolecules or coacervation
5) Solvent evapration
6) Solvent deposition

1) DIRPERSION – BASED PROCESS

a) WET MILLING

Wet milling is an attrition-based process in which the drug is dispersed first in an aqueous-based surfactant solution. The resulting suspension is subjected to wet milling using a pearl mill in the presence if milling media.

b) HIGH – PRESSURE HOMOGENIZATION

High-pressure homogenization is based on the principle of cavitations (i.e., the formation, growth, and implosive collapse of vapor bubbles in a liquid. In this process, a drug pre-suspension (containing drug in the micrometer range) is prepared by subjecting the drug to air jet milling in the presence of an aqueous surfactant solution. The main advantage of high-pressure homogenization is suitable for both large and laboratory scale production, because high-pressure homogenizers are available in various sizes. In addition, homogenization creates negligible nanoparticle contamination, which is one of the most important objectives of a nanoparticle production. A limitation of this process is that the pressure used is so high that in some case, the crystal structure changed.

c) EMULSIFICATION TECHNOLOGY

Emulsification also can be used to prepare nanoparticle suspensions. In this method, the drug solution in an organic solvent is dispersed in the aqueous phase containing surfactant. This step is followed by the evaporation of organic solvent under reduced pressure, which results in the preparation of drug particles to form a nanoparticle suspension which is stabilized by the added surfactant. The use of micro emulsion as templates for producing drug nanosuspension .

d) PRECIPITATION – BASED PROCESS

a) SPRAY FREEZING INTO LIQUID (SFL)

In this process , developed at the university of Texas at Austin (Austin, TX) and commercialized by Dow chemical company (midland, MI) , an aqueous, organic, or aqueous co-solvent solution, aqueous-organic emulsion , or drug suspension is atomized into a cryogenic liquid such as liquid nitrogen to produce frozen nanoparticles which are subsequently lyophilized to obtain free flowing powder.

b) EVAPPORATIVE PRECIPITATION INTO AQUEOUS SOLUTION (EPAS)

The EPAS process also was developed by the University of Texas at Austin and commercialized by Dow chemical company. In this process, the drug solution on boiling liquid organic solvent is heated under pressure to a temperature above the solvent normal boiling point and then atomized into a heated aqueous solution containing stabilizing surfactant.

c) RAPID EXPANSION FROM A LIQUID – GAS SOLUTION (RESS)

In an RESS process, a solution or dispersion of phospholipids or other suitable surfactant in the supercritical fluid is formed. Then, rapid nucleation of drug is induced in the supercritical fluid containing surfactant. This process allows rapid, intimate contact of the drug dissolved in supercritical fluid and the surfactant which inhibits the growth of the newly formed particles.

d) PRECIPITATION WITH A COMPRESSED FLUID ANTI – SOLVENT (PCA)

In the PCA process (patented by RTP pharmaceuticals and licensed to skye pharma PLC (London, UK), supercritical carbon dioxide is mixed with organic solvent containing drug compounds. The solvent expands into supercritical carbon dioxide, thus increasing the concentration of the solute in the solution, making it supersaturated, and causing the solute to precipitate or crystallize out of solution.

e) INTERFACIAL POLYMERIZATION

In interfacial polymerization process takes place at the interface between two immiscible phases. The monomer and the lipophillic drug are dissolved in oil or in benzyl benzoate. The organic phase is slowly added through a small tube under permanent stirring at PH 6 to the aqueous phase containing a surfactant.

f) NANOPARTICLES FORMULATION BY DESOLATION OF MACROMOLECULES OR COACERVATION

Macromolecules in solution have an expanded swollen random structure. To better the solvent is more diluted leads to more swelling of the molecule. Addition of a de-solvating agent (non solvent), charge changes or PH changes reverse this process and result in the de-salvation of the macromolecules. This process is commonly known as co-acervation, a new phase (coacervate phase) is formed . The coacervate phase when treated with a cross linking aldehyde produce nanoparticles of the macromolecule.

g) SOLVENT EVAPORATION

In this process, a polymer is dissolved together with a hydrophobic drug in a volatile and water immiscible organic solvent. The latter is dispersed in water by stirring and evaporated under reduced pressure. The polymer precipitates in the form of microspheres containing the drug finely dispersed in the polymer matrix. Nanoparticles can be formed of the organic mixture is emulsified to form submicron size droplets, using a dispersing agent and high energy homogenization.

h) SOLVENT DEPOSITION

In this process polymers as well as phospholipids are dissolved in acetone. A solution of the drug in benzyl benzoate is than added to the organic phase, and this mixture is subsequently poured into water containing 0.5% polymer 188 under moderate stirring. Nanocapsules with an oily core are formed instantaneously. This suspension then has to be concentrate to about 10ml final volume by evaporation of acetone and partial removal of water under reduced pressure.

1.11. APPLICATION OF NANOPARTICLES[7]

- ✓ **Nano medicines**: nanodrug , medical devices , tissue engineering etc.,
- ✓ Ceramic based nanoparticles for entrapping therapeutic agent for photodynamic therapy, in this method of the photosensitive drug/dye is entrapped with ceramic carrier . These ceramic nanoparticles are widely used for skin and therapeutic purpose.
- ✓ The thermo sensitive nanoparticle may be used for selective release of the content after specific localization like photodynamic therapy.

Materials: nanoparticles, carbon nanotubes, biopolymers, paints, coating .

Table 5. Application of nanoparticles

S.NO	APPLICATION	MATERIALS	PURPOSE
1	Intracellular targeting	Poly (alkylcyanocrynate) poly ester, nanoparticle with anti-neoplastic or antiviral agent	Target reticulo-endothelial system for intracellular infection
2	Vaccine adjuvant	Poly(methylmethacrylate) nanoparticles with vaccines oral and intramuscular immunization	Enhances immune response , alternate acceptable adjuvant
3	DNA delivery	DNA – gelatin nanoparticles, DNA-chitosan nanoparticles, PDNA-poly (DL-lactide-co-glycolite) nanoparticle	Enhanced delivery and significantly higher expression levels
4	Cancer therapy	Poly(alkylcyanoacrylate) nanoparticles with anticancer agents	Targeting reduced toxicity, enhanced uptake of antitumor agents , improved in-vitro and in-vivo stability
5	Per oral absorption	Poly (methacrylate) nanoparticles with proteins and therapeutic agents	Enhanced bioavailability protection from G.I.T

1.12. DIURETICS [9,10]

Definition: Diuretic agents are drugs that increase renal excretion of water and solutes (mainly sodium salt)

Mechanism of diuretics:

- Diuretic act by inhibiting sodium reabsorption in the renal tubules, thereby increasing urinary sodium , and consequently , water loss,
- Agents differ with respect to the specific tubular ion transport system they inhibit,
- Site of action within the nepron
- Natriuretic efficacy
- Pharmacological effects
- Clinical indications
- Site of action located on the luminal surface of the tubule
- Extensively bound to serum albumin
- Transported into the proximal tubule lumen by active secretion
- Organic acid secretory pathway : thiazides , loop diuretics , acetazolamide
- Organic base secretory pathway : potassium-sparing diuretics
- Exception : spiranolactone and eplerenone enter renal tubules from plasma

Major purpose of diuretic therapy are to decrease fluid volume of the body, and to adjust the water and electrolyte balance .

Diuretics are often used in the management of pathological conditions such as edema (e.g. In congestive heart failure and certain renal disease) and hypertension .

Diuretics may be used in surgery to reduce blood pressure and swelling, (mannitol, an osmotic diuretic may be used to reduce swelling in the brain for some neurosurgical procedure)

Table 6. Types of diuretics :

Type	Example	Site of action	Mechanism
Carbonic anhydrase	Acetazolamide	Proximal	Inhibition of

inhibitors		tubule	CA
Osmotic	Mannitol	Loop of Henle ,proximaltubule	Osmatic action
Loop diuretics	Furosemide	Loop of Henle	Inhibition of Na^+-k^+-$2Cl^-$ Symport
Thiazide	Hydrochlorothiazide	Distal convoluted tubule	Inhibition of Na^+-Cl^- symport
Potassium-Sparing diuretics			
1) Na^+ channel inhibitors	Triamterene,	Cortical collecting tubule	Inhibition of Na^+ channel
2) Aldosterone spiranolactone	Amiloride	Cortical collecting tubule	Inhibition of Na^+ channel

Adverse effects of diuretic drugs:

Hypotension and orthostasis, Volume depletion, Electrolyte disorder, Hypokalemia,Hypomagnesemia, Hyponatremia, Hypocalcemia (loopdiuretics), Hypocalcemia (thiazides), Hyperuricemia, Hyperglycemia, glucose intolerance, Dyslipidemia, Photosensitivity and skin reaction, Oxotoxicity, Interstitial nephitis.

2.0 LITERATURE REVIEW

A.umar faruksha[11]et al.,(2010) formulated nanoparticle for Pioglitazone Hydrochloride having low solubility and high permeability were prepared by solvent evaporation displacement method. The dissolution profile of all formulations was fitted to zero order, Higuchi and Korsemayer Peppas models to ascertain the kinetic modeling of drug release. The prepared formulations were further evaluated for drug content, drug- excipient interactions, surface morphology by SEM, differential scaning calorimetry (DSC), Zetapotential. All formulations were found to significantly influence the particle size and entrapment efficacy. the *in-vitro* drug release profile showed that the suitability of chitosan loaded nanoparticles in sustaining Pioglitazone release for prolonged time.

Heati H[12] et al.,(1997) prepared the solid lipid nanoparticles (SLNs) using Trilaurine (TL) as the SLN core and phospholipid (PL) as coating . The stability of SLNs formulations containing AZT-P was studied at different temperatures. Drug retention and mean particle diameter of SLNs were determined after autoclaving during temperature stability testing and after lyophilization(with or without cryoprotective sugars) and reconstitution. There were no significant changes in the mean diameter and the zeta potential of SLNs after autoclaving (121° C for 20 mins). SLNs containing AZT-P can be autoclaved, lyophilized and reconstitution without significant changes in SLN diameter.

Jin – ki kim [13] et al.,(2010) developed Lipid nanoparticles of Itraconazole (ITZ) to proved the controlled release of ITZ as well as improve the solubility of ITZ by high-pressure homogenization method. The particle size and poly dispersity index (PI) of lipid nanoparticles were below 280nm and 0.2nm respectively. Zeta potential and incorporation efficiencies of lipid nanoparticles were around – 30m V and above 80%, respectively. SEM, DSC, and PXRD revealed that ITZ in lipid nanoparticles exist in an amorphous state. Release rates were increased as the amount of liquid lipid in lipid core increased. The release of ITZ from lipid nanoparticles could be controlled by modulation of the amount of liquid lipid in lipid core.

C.X. Song [14] et al.,(1997) formulated various drug – loaded (lactic-co-glycolic acid) (PLGA) nanoparticles (NP) using an emulsification / solvent evaporation technique. Different emulsion systems were employed according to the solubility of individual drugs. Bovine serum albumin was model protein during loaded on nanoparticles 10% to 30%. Typical particle size rearranged from 60-200nm with 80% of the particles in the range of 70-165 nm. The *invitro* release rate for albumin was dependent upon the molecular weight (MW) of PLGA. Nanoparticles with smaller mean size (100nm vs. 266nm) and lower drug loading (13.1% vs. 20.7%) resulted in higher arterial uptakes compared to nanoparticles of larger size and higher drug loading. A wide variety of water soluble and insoluble bioactive agents can be incorporated into PLGA nanoparticles with a high efficiency and adjustable drug loading.

Einat cohen-sela [15] et al.,(1994) described about double system with a partially water-soluble organic solvent, could result in better encapsulation yield of hydrophilic molecules in nano-sized NP, and the utilization of both biocompatible surfactant and solvents. As a model drug they used Alendronate, a hydrophilic low molecule weight bio phophosnate. The NP preparation technique of double emulsion solvent diffusion (DES-D) method resulted in improved formulation characteristics including smaller size, lower size distribution, higher encapsulation yield and more biocompatible ingredients in comparison to classical methods. The utilization of partially water-miscible organic solvent (ethyl acetate) enabled rapid diffusion through the aqueous phases forming smaller NP.

D.Dhachinamoorthi[16] et al.,(2001) prepared Acyclovir loaded chotosan nanoparticles were by ionic gelatin of chitosan with sodium tripolyphosphate (0.25%) prepared in the presence of Tween 80 (0.5%) as a re-suspending agent to prevent aggregation, at ambient temperature while stirring. The DSC thermogram was no chemical interaction between acyclovir and chitosan. The mean particle size, morphological characteristic and surface property of the nanoparticle appear to depend on concentration of Acyclovir, loaded chitosan nanoparticles. The effective use of acyclovir loaded chitosan nanoparticles as a controlled release preparation for treatment of ocular viral infections.

Xinyi Gu [17] et al.,(2005) studied that many drugs, due to their hydrophilicity, have poor loading in nanoparticles, which has been limited drug delivery. Charge-charge interaction may be effective for improving loading where charges in nanoparticles attract oppositely charged drug molecules. A new strategy, incorporation of charged hydrophobic excipients into nanoparticles followed by drug loading incubation of nanoparticles in the presence of drug solution, may effectively increase drug loading. Ion pairing between alkyl sulfates and doxHCL yielded hydrophobic complexes based on solubility and partition coefficient determination and indicated favorable incorporation into hydrophobic nanoparticle cores. However, encapsulation into nanoparticle failed due to poor complex solubility in organic solvents and no significant improvement in drug loading after incorporation.

Kharia Ankit Anand [18] et al.,(2008) described in recent years scientific and technological advancement have been made in the rate controlled oral drug delivery system by overcoming advertise, such as short gastric residence time. Various polymers have been used in the formulation of stomach specific mucoadhesive nanoparticles for drug delivery to increase therapeutic benefit, while minimizing side effects. Discussed about concept of gastric emptying, absorption window, potential drug candidates, technological development evaluation and applications for stomach-specific mucoadhesive nanoparticles .

Jundong Dai [19] et al.,(2004) prepared the Cyclosporine pH sensitive NP using poly (methacrylic acid and methacrylate) copolymer by quasi-emulsion solvent diffusion technique and evaluated the characterization, dispersion state of cyclosporine at the surface or inside the polymer matrices of the NP s *invitro* release the PH-sensitive NPs can be designed as new Cyclosporine carriers with improved oral bioavailability of Cyclosporine.

Devarajan PV[20] et al.,(2007) formulated the Gliclazide Loaded Eudragit nanoparticles (Eudragit L 100 and Eudragit RS) as a sustained release carrier by controlled precipitation method for Eudragit L 100 nanopatricles, solvent evaporation method for Eudrgit RS nanoparticles. The influence of various formulations factors (stirring speed, drug polymer ratio, homogenization & addition of surfactant) on

particles size drug loading & encapsulation efficiency. Eudragit nanoparticles had decreased time, enhanced bioavailability & sustained activity. Hence the Eudragit nanopaticles could reduce dose frequently, decrease side effect & improve patient compliance.

Andre .A. Onischuk [21] et al.,(2008) practiced the respiratory system provides entry for drug nanoparticles to cure systemic disease. The modern devices that are available on the market of therapeutic Aerosol delivery systems have a number of disadvantages. There remains a need for an alternative means that is low cost, convenient, and capable of producing small-sized particles. On the other hand, one -third of the modern drugs are poorly water soluble. Many currently available injectable formulations of such drugs can cause side effects that originate from deterginate and other agents used for their solubilization. The aerosol lung administration may be a good way for delivery of the water-insoluble drugs. Indomethacin nanoaerosol formaed and its anti-inflammatory effect of the outbred male mice was examined. The aerosol lung administration experiments were carried out in the whole-body exposure champer. The anti-inflammatory action and pulmonary effects caused by the inhalation of Indomethacin nanoparticles more effective than peroral treatment. The aerosol route required a therapeutic dose six orders of magnitude less than that for peroral administration.

F. De Jaeghere [22] et al.,(2009) incorporated the poorly water soluble HIV – 1 protease inhibitors into PH – sensitive nanoparticles & macroparticles made of poly (methcrylic acid – co – ethylcrylate) copolymer Eudragit L100-55 . Nanoparticles were characterized in terms of morphology, size – distribution, drug loading, production yield, *invitro & invivo* release studies in beagle dogs. potential of PH-sensitive particles for the oral delivery of HIV – 1 protease inhibitors with low water solubility.

S. Ramesh [23] et al.,(2010) prepared the nanoparticles by using sepia (cuttle fish ink) Ciprofloxacin Hcl was used as a model drug . The prepared formulations were subjected to different *invitro* analysis such as content analysis, particle size analysis, Zeta potential analysis, *invitro* drug release and stability studies. The particle size is about 500nm, the drug content is in the range of drug release was about 85%. CN3

formulations were found to be the best formulation with higher cumulative percentage of drug release.

Partha Saha [24]et al.,(2011) developed Ampicillin Trihydrate-loaded chitosan nanoparticles by modified ionic gelation method and evaluate their Antimicrobial activity. Parameters such as zeta potential, polydispersity, particle size, entrapment efficiency and *invitro* drug release of the nanoparticles were assessed for optimization. Concentration of 0.35%w/v of chitosan and 0.40%w/v sodium tripolyphosphate (TPP) and a sonication time of 20 min constituted the optimum conditions for the preparation of the nanoparticles . The *invitro* release data showed an initial burst followed by slow sustained drug release. Polymer and cross linking agent concentration and sonication time are rate-limiting factors for the development of the optimized formulation. The chitosan nanoparticles developed would be capable of sustained delivery of Ampicillin Trihydrete.

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Mohamed Vaseem[25]et al.,(2005) synthesized Zno nanoparticles by either sol-gel method or hydrothermal method. Synthesis of Zno nanoparticles in the solution requires a well defined shape of Zno nanoparticles . And reported room-temperature, organometallic synthesis of Zno nanopaticles of controlled shape and size an solution. The decomposition of Organomatallic precursor to the oxidized material in air. This preparation was characterized by X-ray diffraction (XRD & TEM conformed as agglomerated Zno nanoparticle with a zincite structure having lack of defined shape and size .

Eliao Leo[26]et al.,(2009) prepared the PLA nanoparticles containing a lipo-philic drug in water-soluble form (AD6) & in water insoluble form (AD6-acid) by nanoprecipitation method the nanoprticles were subjected to evaluation such as drug content, mean particles size, *invitro* release. They changing the pH of the aqueous phase, the drug content dramatically increased .

Kathleen Dillen[27]et al.,(2008) developed the PLGA nanoparticles incorporating Ciprofloxacin HCL by W/O/W emulsification solvent evaporation method. The effects of different preparation factors of the nanoparticles physiochemical properties like particle size, zeta potential, drug loading efficiency and drug release and they

concluded that the homogenization decrease the particle size and release rate of ciprofloxacin, but increased entrapment efficiency, addition of boric acid to the inner water phase, increase drug release rates but only after 6.5 hrs. Also the effect of gamma radiation on the particle size and drug release was evaluated. Differential scanning colorimetry & x-ray diffraction analysis were performed.

Hannele Eerikanen[28] et al.,(2010) described polymeric drug containing nanoparticle were prepared using a novel aerosol flow reactor method. The polymeric drug containing nanoparticle prepared consist of a poorly water soluble corticosteroid Beclomethasone Bipropionate and polymeric materials Eudragit E 100 or Eudragit L 100. The novel method used in this preparation allows synthesis of nanoparticle directly as dry powder. The nanoparticle contain various ratio of drug and polymer & use of any additional stabilization materials is avoided. All the nanoparticle produced, regardless of particle composition, has geometric number mean diameter of approximately 90nm and were spherical showing smooth surfaces. The drug was more currently polymeric matrix dispersed in the amorphous polymeric matrix of the nanoparticle and drug crystallization was not observed, when the grass transsitition temperature of the polymer .

Rubiana[29] et al.,(2012) prepared spherical nanoparticulate drug carriers made of Poly (d,1-lactide-*co*-glycolide) acid with controlled size Praziquantel is a hydrophobic molecule, was entrapped into the nanoparticles with theoretical loading varying from 10 to 30% (w/w). The effects of some process variables on the size distribution of nanoparticles prepared by emulsion-solvent evaporation method. The results show that sonication time, PLGA and drug amounts, PVA concentration, radio between aqueous and organic phases, and the method of solvent evaporation have a significant influence on size distribution of the nanoparticles.

Mainardes Rubiana Mara[30] et al.,(2010) developed PLA and PLA/PEG blend nanoparticles containing Zidovudine and their uptake by polymorphonuclear leucocytes were studied in *invitro* release. The cells were isolated from rat peritoneal exudates and their activation by nanoparticles was measured by luminol-dependent chemiluminescence and microscopical analysis. The phagocytosis depended on the PEG and its radio in the blend, the results showed that the PLA nanoparticles were

more efficiently phagocytosed than PLA/PEG blends. The blend with the highest PEG proportion did not prevent phagocytosis, indicating that the steric effect of PEG was concentration dependent.

Esko I . Kavappinen[31] et al.,(2012) focused on the development of nanoparticle systems for Anti-microbial drug to kill or inhibit the growth of microbes such as bacteria, fungi & viruses. Even though the therapeutic efficiency of these drugs has been well established inefficient delivery could result in inadequate therapeutic index and local and systemic side effect include cutaneous irritation, peeling, and scaling & gut flora reduction. Nano structured bio-material in nanoparticle in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio , high reactivity & functionalizable structure. These properties can be applied to facilliate the administration in traditional anti-microbial therapeutics. In recent years , encapsulated of Anti-microbial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiviness and minimizes undesirable side effects of the drugs .

Yadav SC[32] et al.,(2010) investigated on Biodegratable polymeric nanoparticles based drug delivery systems nanoparticulate drug delivery system seem to be a viable and promising strategy for the biopharmaceutical industry. It can increase the bio-availability, stability and permeability of many potent drugs which are otherwise difficult to deliver orally. Nanoparticle drug delivery systems will also reduce the drug dosage frequently and will increase the patient compliance. In near future nanoparticulate drug delivery systems can be used for exploiting many biological drugs which have been poorly water solubility, permeability and less bio-availability nanoparticles provide ingenious treatment by enabling targeted delivery and controlled release.

Roberta cavalliTrotta[33] et al.,(1997) prepared (SLN) from three oil in water micro emulsions , where internal phase was constituted of different lipid matrics. The dispersion media were two aqueous solutions of trehalose and pluronic F68 at 2% besides distilled water. SLN were sterilized by autoclaving, were stable during sterilization and maintained a spherical shape and narrow size distribution as confirmed by TEM analysis . SLN dispersion in water did not present nanoparticles

large than after at 4° C for 1 year they were freeze dried sterilization to obtain dry products .

Morteza Azhdar[34] et al.,(2010) prepared Azithromycin nanoparticle by modified solvent diffusion method. The anti-bacterial activities of prepared nanoparticle in comparision with Azithromycin solution were assayed against indicator bacteria of Escharichia coli (PTCC-1330), Haemophilus influenza (PTCC-1623) & streptococcus pneumonia (PTCC-1240) using agar well diffusion. Inhibition zone diameters (IZD) of nano-formulation were compared to the corresponding untreated AZI. Mean inhibitory concentration (MIC) were also determined using serial dilution method in nutrient broth medium. The enhanced anti-bacterial efficacy was more dominant in the gram positive species. The MIC values of nanoparticles against the tested bacteria were reduced 8 times in comparison to those of untreated AZI. An improved potency of AZI nanoparticles which could be attributed to the modified surface characteristic as well as increased drug absorption and uptake.

Jahangiri L et al.,(2013) developed importance and increasing application nanoparticles and their toxicity, the identification effect of nanoparticles on physiological systems are essential. Some studies show magnesium has analgesic effect in some pain models but this evaluation was not carried on nano-Magnesium oxide (MgO) thus, present study was designed evaluation effect of MgO nanoparticles alone and in combination with Ketamine on pain and inflammation model in mice.

Vivek kumar gupta[36] et al.,(2009) formulated Nanoparticle of 5-Flurouracil using chitosan polymer and pregelated using alginate by ionotropic pregelation method. Calcium chloride was also included in the formulation for pregelation of sodium alginate prepared 1% acetic acid solution of chitosan and pre gelation of sodium alginate suspension further cross-linked with gluteraldehyde. Different formulation of nanoparticles were prepared using different concentration of chitosan, stirring speed, time of rotation and polymer to drug ratio in the nanoparticles. The average particle size ranged between 246nm to 620nm. Drug entrapment ranged between 71.9%-89.90%. The drug loaded nanoparticle of 5-flurouracil showed optimum particle size and maximum drug entrapment with drug polymer ratio 05:75, cross-linking agents, stirring speed 800rpm and stirring time 90min.

Peng Guo[37] et al.,(2005) prepared hydrophobic drug nanopaticle by nanoporous membrane extrusion. NME is based on the induced precipitation of drug-loaded nanopaticle at the exists of nanopores. These common hydrophobic drug models (silymarin , β-carotene) were tested. Themmorphology, crystallinity, dissolution profile of resulting nanoparticle. Using NME, the successfully prepared rather uniform drug nanoparticle. These nanoparticle were amorphous and show improved dissolution profile compared with untreated drug powder. NME could be used as general method to produce nanopaticle drug.

Rubina M . Marnardes[38] et al.,(2010) designed nanoparticles for Praziquentel using poly (D , L-lactide-co-glycolide) (PLGA) as a carrier. The effects of some process variables on the size distribution of nanoparticles prepared by emulsion solvent evaporation method. The results showed that sonication time, PLGA and drug amounts, PVA concentration, ratio between aqueous and organic phases and the method of solvent evaporation have a significant influence on size distribution of the nanoparticles.

Swarnali Das[39] et al.,(2011) prepared Am-B loaded Eudragit nanoparticles by A solvent displacement technique . These NPs had a mean size range of 150-290m and a zeta potential of +19-28 mV. Even after 6 months of stability study, results were unchanged the good potential for ocular application. In vitro release studies revealed that a maximum amount of drug was released within 24 hours (60%). The microbial assay showed that the anti fungal activity of drug-loaded NPs was equal to or slightly lower than that of free-AmB solution . In vivo experiments showed that, following topical installing of nanosuspension to a rabbit eye there was no irritation. Eudragit RS 100 nanosuspension may represent an efficacious vehicle to deliver the drug into drug into the eye.

Adlni jino nesalin[40] et al.,(2009) prepared Flutamide nanoparticles by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for the total drug content, loading efficiency, particle size and in vitro drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat gives better sustained release for about 12 hrs compared to other formulations.

J. Vandervoot [41] et al.,(2002) prepared nanoparticles by W/O/W emulsification solvent evaporation method. Poly (vinyl alcohol) is used as stabilizer of the emulsion. The influence of the concentration of PVA and the polymers tested on particle size and zeta potential value was evaluated before and after freeze-drying of the prepared particles. Nanoparticles were obtained with the formulations, however, increased the size of the particles over 1 m. Two exceptions are Poloxamer and Carbopol, which can be considered as valuable alternatives to PVA. Zeta potential values were measured when Poloxamar and Carbopol were employed. The use of gelatin type A made it possible it achieve values.

T. Vetrichelvan[42] **et al**.,(2011) prepared Alginate nanoparticles by in situ nano emulsion polymer cross linking approach. The nano particles were prepared using different ratios of alginates and Abacavir sulfate in the ratios of (1:1, 1:2 and 1:3) The result of ratio 1:3 showed a good encapsulation efficiency of 98.71%. Abacavir sulfate nanoparticles was confirmed by FT-IR, DSC and quantitated by uv prepared nanoparticle appeared spherical with a dense drug core in transmission electron microscopy studies . Hydro dynamic diameter of nanoparticles was 63 +/- 0.235 nm, with a Gaussian distribution and the zeta potential-0.6. The nanoparticle technique developed can be a good choice for the development of sustained antiretroviral drug carrier.

N.Jawahar[43] et al.,(2009) prepared Poly (D,L-Lactide-co-Glycolide) (PLGA) nanoparticles by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic RF-68). The particles were characterized for drug content, particle size and particle morphology by Transmission electron microscope (TEM). In vitro studies were determined by the bulk equilibrium reverse dialysis bag technique. The particle size of the prepared nanoparticles ranged from 200nm to 340nm. nanoparticles of Ramipril were obtained with high encapsulation efficiency (68-75%). The drug release from the Ramipril nanoparticles was sustained in Batch (F3) for more than 24 hrs with 72% drug release. The feasibility of formulating Ramipril loaded PLGA nanoparticles can be used to improve the therapeutic efficacy of Ramipril in the treatment of hypertensive disorder.

Atul Gaikwad[44] et al.,(2010) described about Furosemide loaded Eudragit RS100 nano particles prepared by nanoprecipitation method. The shape of nanoparticles was found to be spherical by scanning electron microscopy study where as size ranging from 163nm to 378nm. FT-IR study confirmed that there was no interaction between drug and polymer. Entrapment efficiency in the range of 14.95-0.06 to 69.73-0.03 W/W. Zeta potential of formulation supports the minimum interaction between the particles. The invitro drug release study revealed that sustained release of some formulation last up to 24 hour.The release followed Higuchi kinetics, which indicates diffusion controlled release pattern of drug.

S.Tamizhrasi[45] et al.,(2009) studied the preparation and evaluation of poly Methacrylic acid nano particles containing Lamivudine in different drug to polymer ratio by nanoprecipitation method. SEM indicates that nano particles have a discrete spherical structure without aggregation. The average particle size was found to be 121 to 403nm. The drug content of the nanoparticles was increasing on the increasing polymer concentration up to a particular concentration. FTIR studies indicate there was no chemical interaction between drug and polymer and stability of drug. The invitro release behavior from all the drug loaded batches was found to be zero order and provided sustained release over a period of 24 hours. The development formulation overcome and alleviates the drawbacks and limitation of Lamivudine sustained release formulations.

Sergio A[46] et al.,(2005) studied the scale up technologies for Ibuprofen loaded nanoparticles by three manufacturing process salting out, emulsification diffusion and nanoprepicitation to pilot scale by increasing 20 fold laboratory –batch volume from 60ml to 1.5L using Eudragit L100-55 as polymer. Influence of the photodynamic condition on the nanoparticles characteristics in the scale up process and concluded that nanoparticles were reproduced well at both scales, however included a slight reduction in the size and drug loading of nanoparticles.

Arvind Gulbake [47]et al.,(2012) described Chitosan nanoparticles (CH-NPs) bearing Mesalazine by ionotropic gelation method and encapsulated in Eudragit S 100 coated pellets for site specific delivery to ulcerative colitis. The CH-NPs were characterized for size and structured using Malvern zetasized and transmission electron spectroscopy (TEM). The average size of the un-coated CH-NPs was about 157.3±

7.1 nm, with the zeta potential of 32.2±2.1nm, suitable for uptake through the colonic mucosa due to their nanosize range and mucoadhesive properties. The invitro drug release from developed formulations was investigated using a USP dissolution rate paddle-type apparatus. Different stimulated gastrointestinal tract fluids. The coated formulation shows no release and un-coated CH-NPs showed 4.98±0.24%, of MSZ in SGF (PH 1.2). The release of drug from coated nanoparticles was PH responsive. At the end of MSZ was released 24 hrs 69.24 ± 3.4% , & 45.26 ± 2.4 % of MSZ was released CH-NPs . The MSZ and pellets of CH-NPs and EC-CH-NPs bearing MSZ were separately administrated orally at the dose of 50mg/kg body weight to albino rats and evaluated for anti-ulcerogenic activity. Anti-ulcerogenic activity studies on albino rats were done for determining the effectiness of formulations in the management of ulcerative colitis.

Rakesh kumar sharma[48] et al.,(2013) developed Metformin solid lipid nanoparticles (M-SLN) and incorporate it in the transdermal patches. M-SLN was evaluated for Particle size, Zeta potential, Patches were evaluated by Ex-vivo skin permeation studies. M-SLN was prepared by solvent diffusion technique using propylene glycol (solvent), polymethacrylic acid (polymer) and Soya lecithin (lipid base). The particle size of M-SLN varied among the formulation due to variation in the composition of formulations. Zeta potential of best formulation was found to be +27mV. SEM and TEM indicate discrete spherical structure without aggregation. Drug content was found to be 1.45mg/patch. Transdermal delivery of M-SLN is a safe, painless and cost effective drug delivery system for diabetes patients.

Waree Tiyaboochai [49] et al.,(2001) Studied that Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Their sub-micron size not only suitable for parenteral application, but also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa, which arenon-invasive route. The application for mucosal delivery also facilitated by chitosan absorption enhancing effect. Furthermore, chitosan nanoparticles also showed to be a good adjuvant for vaccines.

Ravindra Kulkarni [50] et al.,(2010) developed and formulate the sustained release Glipizide loaded nanoparticles and evaluate it. Emulsification-solvent evaporation

technique. The optimized nanoparticles formulation were studied for FT-IR, particle size, zeta potential, encapsulation efficiency, XRD, in vitro release study and in vivo evaluation etc. The effects of dependent variables drug-polymer ratio (X1) and surfactant concentration (X2) on particle size and encapsulation efficiency were studied. The drug and polymer were not interacting with each other. The particles were smooth, spherical and and homogeneous external aspects. The crystallinity of nanoparticles was less than pure Glipizide. The selected formulation for dissolution study shows 209.6 nm size and 95.66±1.70 percent encapsulation efficiency. In vitro release was found to be much sustained up to seven days (64.79±2.68) and follow first order kinetic. The sustained release naoparticles decreased the blood glucose level up to 132.66±9.83 mg/dL in seven days study period. The sustained release nanoparticles of glipizide could be able to manage type II diabetes mellitus with reduced dose frequency, decreased side effects and improve patient compliance.

Thommey P Thomas[51] et al.,(2012) described nanoparticle drug delivery might improve the therapeutic response to anticancer drugs and allow the simultaneous monitoring of drug uptake by tumors. Acetylated dendrimers were conjugated to folic acid as a targeting agent and then coupled to either Methotrexate or Tritium and either Fluorescein or 6-Carboxytetramethylrhodamine. These conjugates were injected i.v. into immunodeficient mice bearing human KB tumors that overexpress the folic acid receptor. In contrast to nontargeted polymer, folate-conjugated nanoparticles concentrated in the tumor and liver tissue over 4 days after administration. The tumor tissue localization of the folate-targeted polymer could be attenuated by prior i.v. injection of free folic acid. Targeting Methotrexate increased its antitumor activity and markedly decreased its toxicity, allowing therapeutic responses not possible with a free drug.

Amrita Dikpati [52] et al.,(2013) explained the use of nanoparticles as carrier system for drugs to cross the barriers of the CNS. Different types of nanoparticles, their methods of production and methods for the characterization of nanoparticles have been discussed. There is a need to develop effective, preferably bio degradable, as well as safe nanoparticulate drug delivery systems, which is to be developed, is discussed under future prospect. A list of research work conducted in the field of CNS targeted drug delivery using nanoparticles has been provided encompassing the work

of research groups in the above review article along with recent patents and FDA approved products.

Sarah D.brown[53]et al.,(2010) studied the platinum-based anticancer drugs Cisplatin, Carboplatin, and Oxaliplatin are an important component of chemotherapy but are limited by severe dose-limiting side effects and the ability of tumours to develop resistance rapidly. These drugs can be improved through the use of drug-delivery vehicles that are able to target cancers passively or actively. The active component of the anticancer drug oxaliplatin to a gold nanoparticle for improved drug delivery. Naked gold nanoparticles were functionalized with a thiolated poly(ethylene glycol) (PEG) monolayer capped with a Carboxylate group. The platinum-tethered nanoparticles were examined for cytotoxicity, drug uptake, and localization in the A549 lung epithelial cancer cell line and the colon cancer cell lines HCT116, HCT15, HT29, and RKO. The platinum-tethered nanoparticles demonstrated as good as, or significantly better, cytotoxicity than oxaliplatin alone in all of the cell lines and an unusual ability to penetrate the nucleus in the lung cancer cells.

May D wang[54]et al.,(2007) developed Cancer nanotechnology is currently under intense development for applications in cancer imaging, molecular diagnosis and targeted therapy. The basic rationale is that nanometer-sized particles, such as biodegradable micelles, semiconductor quantum dots and iron oxide nanocrystals, have functional or structural properties that are not available from either molecular or macroscopic agents. When linked with biotargeting ligands, such as monoclonal antibodies, peptides or small molecules, these nanoparticles are used to target malignant tumours with high affinity and specificity. In the 'mesoscopic' size range of 5–100 nm in diameter, nanoparticles also have large surface areas and functional groups for conjugating to multiple diagnostic (e.g., optical, radioisotopic or magnetic) and therapeutic (e.g., anticancer) agents. Recent advances have led to multifunctional nanoparticle probes for molecular and cellular imaging, nanoparticle drugs for targeted therapy, and integrated nanodevices for early cancer detection and screening. These developments have opened exciting opportunities for personalized oncology in which cancer detection, diagnosis and therapy are tailored to each individual's molecular profile, and also for predictive oncology, in which genetic/molecular information is used to predict tumor development, progression and clinical outcome.

Jayanth panyam[55]et al.,(2002) reported the rapid (<10 min) endo-lysosomal escape of biodegradable nanoparticles (NPs) formulated from the copolymers of poly(dl-lactide-*co*-glycolide) (PLGA). The mechanism of rapid escape is by selective reversal of the surface charge of NPs (from anionic to cationic) in the acidic endo-lysosomal compartment, which causes the NPs to interact with the endo-lysosomal membrane and escape into the cytosol. PLGA NPs are able to deliver a variety of therapeutic agents, including macromolecules such as DNA and low molecular weight drugs such as Dexamethasone, intracellularly at a slow rate, which results in a sustained therapeutic effect.

Dong M.Shin[56]et al.,(2008) designed to improve the biodistribution of cancer drugs, nanoparticles for optimal size and surface characteristics to increase their circulation time in the blood stream. They are also able to carry their loaded active drugs to cancer cells by selectively using the unique pathophysiology of tumours, such as their enhanced permeability and retention effect and the tumor micro environment. In addition to this passive targeting mechanism, active targeting strategies using ligands or antibodies directed against selected tumor targets amplify the specificity of these therapeutic nanoparticles. Nanoparticles have the ability to accumulate in cells without being recognized by P-glycoprotein, one of the main mediators of multidrug resistance, resulting in the increased intracellular concentration of drugs. Multifunctional and multiplex nanoparticles are now being actively investigated and are on the horizon as the next generation of nanoparticles, facilitating personalized and tailored cancer treatment.

M.A.Khan[57] et al.,(2002) Prepared Nanoparticles (NP) are solid colloidal particles ranging in size from 1 to 1000 nm that are utilized as drug delivery agents. The use of NPs to deliver drugs to the brain across the Blood-Brain Barrier (BBB) may provide a significant advantage to current strategies. The primary advantage of NP carrier technology is that NPs mask the blood-brain barrier limiting characteristics of the therapeutic drug molecule. Furthermore this system may slow drug release in the brain, decreasing peripheral toxicity. Influencing manufacturing factors (type of polymers and surfactants, NP size, and the drug molecule) are detailed in relation to movement of the drug delivery agent across the BBB. Currently, reports evaluating NPs for brain delivery have studied anaesthetic and chemotherapeutic agents.

Physiological factors such as phagocytic activity of the reticuloendothelial system and protein opsonization may limit the amount of brain delivered drug and methods to avoid these issues are also discussed. NP technology appears to have significant promise in delivering therapeutic molecules across the BBB.

Brian G.Trewyn[58] et al.,(2008) Prepared A Boronic acid-functionalized Mesoporous silica nanoparticle-based drug delivery system (BA-MSN) for glucose-responsive controlled release of both insulin and cyclic adenosine monophosphate (cAMP) was synthesized. Fluorescein isothiocyanate-labeled, gluconic acid-modified Insulin (FITC-G-Ins) proteins were immobilized on the exterior surface of BA-MSN and also served as caps to encapsulate cAMP molecules inside the mesopores of BA-MSN. The release of both G-Ins and cAMP was triggered by the introduction of saccharides. The selectivity of FITC-G-Ins release toward a series of carbohydrate triggers was determined to be fructose > glucose > other saccharides. The unique feature of this double-release system is that the decrease of FITC-G-Ins release with cycles can be balanced by the release of cAMP from mesopores of MSN, which is regulated by the gatekeeper effect of FITC-G-Ins. In vitro controlled release of cAMP was studied at two pH conditions (pH 7.4 and 8.5). Furthermore, the cytotoxicity of cAMP-loaded G-Ins-MSN with four different cell lines was investigated by cell viability and proliferation studies.

Sunil A.Agnihotri[59] et al.,(2004) reviewed outlines the major new findings on the pharmaceutical applications of chitosan-based micro/nanoparticulate drug delivery systems published over the past decade. Methods of their preparation, drug loading, release characteristics, and applications are covered. Chemically modified chitosan or its derivatives used in drug delivery research are discussed critically to evaluate the usefulness of these systems in delivering the bioactive molecules. The research activities on chitosan micro/nanoparticulate systems containing various drugs for different therapeutic applications have increased at the rapid rate.

C.Schwarz[60] et al.,(1999) Solid lipid nanoparticles (SLN) are a colloidal carrier system for controlled drug delivery. The lipophilic model drugs Tetracaine and Etomidate were incorporated to study the maximum drug loading, entrapment efficacy, effect of drug incorporation on SLN size, zeta potential (charge) and long-

term physical stability. Drug loads of up to 10% could be achieved whilst simultaneously maintaining a physically stable nanoparticle dispersion. Incorporation of drugs showed no or little effect on particle size and zeta potential compared to drug-free SLN. The optimized production parameters previously established for drug-free SLN dispersions can therefore be transferred to drug-loaded systems to facilitate product development.

Abdul kareem[61] et al .,(2010) studied invitro and invivo pharmadynamic activity of newly synthesized palm oil ester (POES) based nanocream contain Piraxicam for topical delivery. Growing interest in use of topical vehicle systems assist in drug permeation through skin, drug of interest are usually those that are problematic. When given orally, such as Piraxicam, a highly effective anti-inflammatory,anti-pyretic and analgesic but with the side effect of causing gastro intentional ulcers.

3. RESEARCH ENVISAGED

3.1 AIM OF THE WORK

The purpose of this research work was to prepare Furosemide nanoparticles to reduce dosing frequency.

Polymeric nanoparticles have received more attention for preparing sustained release dosage forms because of their inertness, solubility in relatively non-toxic solvent.

Furosemide is a loop diuretic used in the treatment of congestive heart failure, edema, renal failure etc.,

Polymeric nanoparticles are one of the more powerful platforms to attain prolonged release. Since Furosemide has short life time 1 to 1.7 hrs. This research work is focused on the preparation of nanoparticles. By using this Eudragit RL 100 is taken on a release retardant material.

Formulation of Eudragit RL 100 nanoparticles of Furosemide to achieve sustain action, with increasing absorption and thereby to increase it bio-availability.

The slow and constant release of Furosemide from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy.

The developed formulation overcome and alleviation the draw back and limitations of Furosemide sustained release formulation.

3.2 PLAN OF THE WORK

Plan of the work involves the following process:

- Pre formulation studies for raw materials observed.
- Formulation of Furosemide loaded Eudragit RL100 nanoparticles can be prepared by solvent evaporation method.
- FT-IR study will be use for comparison of nanoparticles of preparation with its raw materials.
- The optimized formulation will be selected based on the results of following parameters. The prepared nanoparticles can be evaluated by following chemical characteristics:
- Drug content determination.
- Drug entrapment efficiency.
- In-vitro drug release of formulated nanoparticles .
- The morphology of nanoparticle was by scanning electron microscopy (SEM)
- Zeta potential analysis of the optimized formulation.
- Drug release kinetic study
- Stability studies for the optimized formulation at different temperature.

4.0 METHODOLOGY

Table 7. INSTRUMENTS AND MATERIALS USED:

S.NO	EQUIPMENTS	SOURCE
1	Vortex Mixer	Remi motors Ltd, Mumbai.
2	Rotary flash evaporator	Equitron, Mumbai.
3	Probe sonicator	Bandelin, Germany.
4	Magnetic stirrer	Remi motors Ltd. Mumbai.
5	Single beam UV spectrophotometer	Shimadzu corporation, Japan.
6	Electronic balance	Shimadzu, Singapore.
7	Stability chamber	Osword, Mumbai.
8	Ultra centrifugation	Remi motors Ltd, Mumbai.
9	pH – meter	Elico Pvt, Chennai.
10	FTIR spectroscopy	Perkin Elmer, Germany.
11	Autoclave	Kemi chem., India.
12	Double beam UV spectrophotometer	Perkin Elmer, Germany
13	Laminar air flow	Klenzaids, Mumbai.
14	Incubator	M.C.DAAL&CO, Mumbai.
15	Freeze drier	Allied frost, Mumbai.
16	Hot air oven	Biochemicals, Mumbai.
17	Membrane filter	Gotting Ltd, Germany
18	ZEISS	Oberkochen, Germany.
19	Malvern	RHS Malvern, United kingdom.

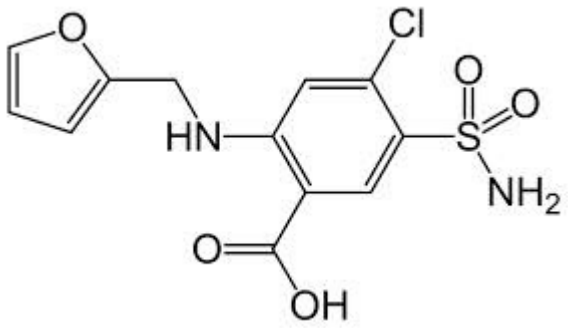
Table 8. MATAERIALS USED

MATERIALS	SOURCE
Furosemide	gsk pharma, Mumbai.
Eudragit RL 100	Micro labs ,Hosur.
Sodium dodecyl sulphate	S.D.Fine chemicals,Boisar.
Ethanol	S.D.Fine chemicals,Boisar.
Potassium dihydrogen phosphate	S.D.Fine chemicals,Boisar.
Disodium hydrogen phosphate	S.D.Fine chemicals,Boisar.
Sodium Hydroxide	S.D.Fine chemicals,Boisar.

4.1 DRUG PROFILE[62]

FUROSEMIDE:

Structure:



Molecular formula:

$C_{12}H_{11}ClN_2O_5S$

Chemical name:

4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoyl benzoic acid

Molecular weight:

330.745 g/mol

Mechanism of action:

Furosemide, like other loop diuretics, acts by inhibiting NKCC2, the luminal Na-K-2Cl symporter in the thick ascending limb of the loop of Henle. The action on the distal tubules is independent of any inhibitory effect on carbonic anhydrase or aldosterone; it also abolishes the corticomedullary osmotic gradient and blocks negative, as well as positive, free water clearance.

Because of the large NaCl absorptive capacity of the loop of Henle, diuresis is not limited by development of acidosis, as it is with the carbonic anhydrase inhibitors. By inhibiting the transporter, the loop diuretics reduce the reabsorption of NaCl and also diminish the lumen-positive potential that derives from K+ recycling. This electrical potential normally drives divalent cation reabsorption in the loop, and by reducing this potential, loop diuretics cause an increase in Mg2+ and Ca2+ excretion.

Prolonged use can cause significant hypomagnesemia in some patients. Since Ca2+ is actively reabsorbed in the distal convoluted tubule, loop diuretics generally do not cause hypocalcemia.

Additionally, Furosemide is a noncompetitive subtype-specific blocker of GABA-A receptors. Furosemide has been reported to reversibly antagonize GABA-evoked currents of α6β2γ2 receptors at μM concentrations, but not α1β2γ2 receptors. During development, the α6β2γ2 receptor increases in expression in cerebellar granule neurons, corresponding to increased sensitivity to furosemide.

Pharmacokinetics:

Molecular weight (Daltons) 330.7

Protein binding 91–99%

Excreted unchanged in urine 80–90%

Volume of distribution (L/kg) 0.07–0.2

Half-life – normal/ESRF (hrs) 0.5–2/9.7

Drug interactions:

Potentially hazardous interactions with other drugs:

Analgesics: increased risk of nephrotoxicity with NSAIDs; antagonism of diuretic effect with NSAIDs

Anti-arrhythmics: risk of cardiac toxicity with anti-arrhythmics if hypokalaemia occurs; effects of Lidocaine and Mexiletine antagonised

Antibacterials: increased risk of ototoxicity with Aminoglycosides, Polymyxins and Vancomycin; avoid concomitant use with Lymecycline

Antidepressants: increased risk of hypokalaemia with Reboxetine; enhanced hypotensive effect with MAOIs; increased risk of postural hypotension with tricyclics

Anti-epileptics: increased risk of hyponatraemia with Carbamazepine

Antifungals: increased risk of hypokalaemia with Amphotericin

Antihypertensives: Enhanced hypotensive effect; increased risk of first dose hypotensive effect with alpha-blockers; increased risk of ventricular arrhythmias with Sotalol if hypokalaemia occurs

Antipsychotics: Increased risk of ventricular arrhythmias with Amisulpiride, Sertindole or Pimozide (avoid with Pimozide) if hypokalaemia occurs; enhanced hypotensive effect with Phenothiazines

Atomoxetine: Hypokalaemia increases risk of ventricular arrhythmias

Cardiac glycosides: Increased toxicity if hypokalaemia occurs

Ciclosporin: variable reports of increased nephrotoxicity, ototoxicity and hepatotoxicity

Lithium: risk of toxicity.

Route:

IV peripherally or centrally, IM, oral.

1 hour; not greater than 4 mg/minute

250 mg to 50 ML sodium chloride 0.9% or undiluted via CRIP (Cysteine-rich intestinal protein)

Increased danger of ototoxicity and nephrotoxicity if infused at faster rate than approximately 4 mg/min.

Protect from light

Medical uses:

Furosemide is primarily used for the treatment of hypertension and edema. It is the first-line agent in most people with edema caused by congestive heart failure. It is also used for hepatic cirrhosis, renal impairment, nephrotic syndrome, in adjunct therapy for cerebral/pulmonary edema where rapid diuresis is required (IV injection), and in the management of severe hypercalcemia in combination with adequate rehydration.

Doses:

Hypertension:

Adult: 40-80 mg daily

Odema associated with heart failure:

Adult: Initially 20 mg daily or 40 mg once daily

Severe case: 600 mg

Child: 1-3 mg/kg daily

Adverse effects:

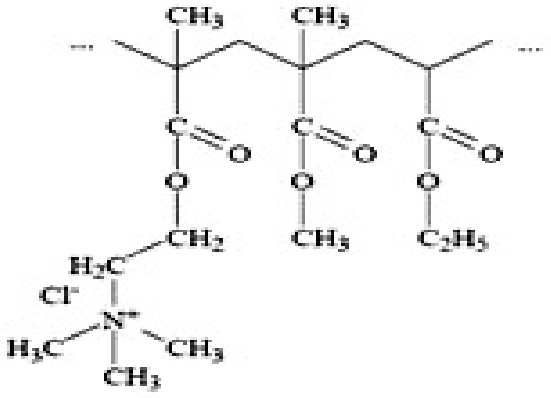
Although disputed, it is considered ototoxic: "usually with large parenteral doses and rapid administration and in renal impairment". Furosemide also can lead to gout caused by hyperuricemia. Hyperglycemia is also a common side effect.

The tendency, as for all loop diuretics, to cause low potassium levels (hypokalemia) has given rise to combination products, either with potassium itself (e.g. Lasix-K) or with the potassium-sparing diuretic Amiloride (Co-Amilofruse).

4.2 POLYMER PROFILE[63]

EUDRAGIT RL 100

Ammonia-methacrylate polymers, consist of fully polymerized copolymers of acrylic acid and methecrylic acid ester with a low content of primary amino group as described by NF XVII.



Molecular weight: 150.000

Functional category: Film former, Tablet diluents

Application in pharmaceutical formulation and technology:

Used to form water insoluble film or film coats for sustained release product, these are also permeable to solvents .

Description:

It is referred as ammonia methacrylate co polymer in the USPNF mono graph, are co-polymer synthesized from acrylic acid and methacrylic acid ester having 100% of functional quaternary ammonium group. The ammonium group is present as salt, and give rise to pH-independent permeability of polymer and it is water insoluble, and film prepared from Eudragit L 100 is freely permeable to water.

Chemical Name:

Poly (ethyl acrylate, methyl methacrylate, triethylamino ethyl methacrylate chloride).

Solubility in various solutions:

Soluble in various alcohols like ethanol, methanol, and propan-2-ol, dichloromethane, solvent ethyl acetate. Insoluble or immiscible in petroleum ether and water.

Plasticizer:

This includes dibutyl phthalate, poly ethylene glycols, and triethyl citrate .

5.0 EXPERIMENTAL INVESTIGATIONS

5.1 CONSTRUCTION OF STANDARD CURVE FOR FUROSEMIDE

A. By UV spectroscopy Method[64] :

Furosemide is estimated spectrophotometrically at 271nm and it obeys Beer-lambert's Law of 1-10µg/ml.

Determination of absorbance maximum (λ_{max})

Furosemide was dissolved in phosphate buffer saline pH 7.4solution with 20µg/ml concentration was prepared by suitable dilution. The solution was scanned in UV spectrophotometer at 200 to 400nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 271nm[65].The drug was later quantified by measuring the absorbance at 271nm in phosphate buffer saline pH 7.4.

Preparation of pH 7.4 phosphate buffer saline[66]

Disodium hydrogen phosphate 2.38gm, potassium phosphate 0.19gm, sodium hydroxide 8gmweighted and it is transformed into 1000ml volumetric flask and volume is made up with distilled water. The pH was adjusted if necessary.

Preparation of stock solution

Stock solution was prepared by dissolving 100mg of Furosemide drugs in 100ml of solvent medium, so as to get a solution of 1000 µg/ml concentration (primary stock solution) from primary stock solution 1ml was taken in 100 ml standard flask and a diluted to 100 ml with solvent medium PBS 7.4 (secondary stock solution) to get the concentration of 1-10mcg/ml.

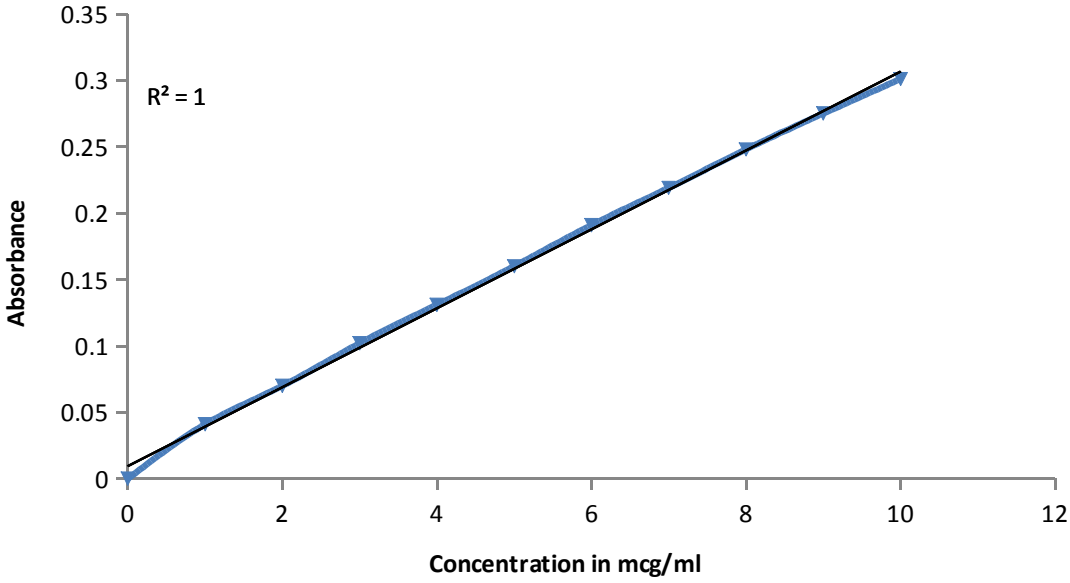
Preparation of standard solution

From the secondary stock solution aliquots from 1 to 10 ml PBS to get the final concentration ranges from 1 to 10 µg/ml. Absorbance of the solution was measured at 271nm UV spctrophotometrically against drug free PBS pH 7.4 media as blank.

Table 9. CALIBRATION CURVE OF FUROSEMIDE

S.NO	Concentration (µg/ml)	Absorbance at 271 nm
1	1	0.041
2	2	0.070
3	3	0.102
4	4	0.131
5	5	0.160
6	6	0.191
7	7	0.219
8	8	0.248
9	9	0.275
10	10	0.301

Fig No:1 STANDARD CURVE FOR FUROSEMIDE



PRE FORMULATION STUDY

5.2 DRUG AND POLYMER COMPATIBILITY STUDY BY FTIR

One of the requirements for the selection of suitable excipient (or) carrier for pharmaceutical formulations is compatibility. Therefore in the present in the present work, a study was carried out using fourier transformed infrared (FT-IR) spectrophotometer (using perkin elmer) to confirm of any possible chemical interaction between the Furosemide and Eudragit RL 100.

Infrared spectroscopy by potassium pellet method was carried out on pure substance (Furosemide, Eudragit RL 100) separately and their physical mixture. They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer.

The spectrum of physical mixture was compared with the original spectra to determine any possible molecular interactions between the drug and polymer. Fourier Transformer Infrared Spectroscopy (FTIR) analysis measure the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The observation of vibration spectrum of encapsulated drug by evaluates the kind of interaction occurring between the drug and polymer.

In the presence work, Furosemide pure drug, pure Eudragit RL 100 was carry out to FTIR and spectra are obtaine . They are compressed under 15 tonnes pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer.

Table 10.IR SPECTRA DATA FOR PURE DRUG FUROSEMIDE

Frequency cm^{-1}	Groups Assigned
508.38-590.11	Cl-Cl – stretching
1672 – 1780	C=O – stretching
1640 – 1680	C=C – stretching
3300 – 3500	NH - stretching
2850 – 2960	CH – stretching

Table 11. IR SPECTRA DATA FOR PHYSICAL MIXTURE

Frequency cm^{-1}	Group Assigned
583.37	Cl – Cl - Stretching
3429.19	OH - Stretching
1718.21	C=O - Stretching
2852.63	CH - Stretching

REPORT:

In FTIR spectra the peaks of physical mixture was compared with the original spectra. Same peaks were observed, there is no possible molecular interaction between the drug and the polymer.

19/09/13
KMCP
FUROSEMIDE
FT-IR SPECTRUM RX1
%T
cm-1
4000.0
3000
2000
1500
1000
500 400.0
3904.95
3629.94
3399.07
3351.93
3284.92
3119.52
2931.82
2556.35
2372.75
1672.46
1591.52
1565.16
1491.94
1448.27
1409.94
1323.60
1242.01
1142.64
1054.62
1015.65
979.70
943.66
920.17
847.21
790.11
743.83
708.38
587.71
581.73
494.37
Spectrum Pathname: C:\PEL_DATA\SPECTRA\FUROUSEMIDE.002
FUROUSEMIDE.pk
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3904.95 50.41 3854.71 49.87 3753.43 48.04 3679.54 48.91 3652.44 48.07
3629.94 48.46 3399.07 26.97 3351.93 26.69 3284.92 27.14 3119.52 38.41
2931.82 37.10 2556.35 40.56 2372.75 44.57 1672.46 13.15 1591.52 22.97
1565.16 16.58 1491.94 36.92 1448.27 39.99 1409.94 35.90 1323.60 20.63
1242.01 21.06 1142.64 12.17 1054.62 40.34 1015.65 43.04 979.70 44.64

Spectrum Pathname: C:\PEL_DATA\SPECTRA\NANOPARTICLES OF FUROUSEMIDE.002

NANOPARTICLES OF FUROUSEMIDE.pk

NANOPARTICLES OF FUROUSEMIDE.002 3601 4000.00 400.00 13.53 38.57 4.00 %T 4 1.00

REF 4000 38.27 2000 30.60 600
3905.26 35.85 3805.88 35.55 3753.87 33.43 3429.19 19.45 2921.14 13.62
2852.63 18.11 2373.86 28.37 1718.21 13.53 1466.46 22.84 1385.15 23.47
1224.27 13.96 1020.15 23.81 835.89 29.02 583.37 27.81

5.3 METHOD OF PREPARATION OF FUROSEMIDE NANOPARTICLES

Solvent Evaporation Method[67]

All batches of nanoparticles were prepared by solvent evaporation method. The required quantity of drug and polymer dissolved in 5ml ethanol (I portion) and 50 mg of sodium dodecyl sulphate dissolved in 5 ml of water, this mixture well dissolved (II portion). Then, the sodium dodecyl sulphate solution mixed with drug, polymer mixture by syringe. This mixture was homogenized by vortex mixture for 1 min and then sonicated set at 90W of energy output for size reduction. Then nanoparticles were collected after solvent drying by flash evaporator.

Table 12. Method of preparation of Furosemide nanoparticles

S.NO	Formulation code	Drug (Furosemide) in mg	Plymer Eudragit RL 100
1	F1	20	10
2	F2	20	20
3	F3	20	30
4	F4	20	40
5	F5	20	50
6	F6	20	60
7	F7	20	70
8	F8	20	80
9	F9	20	90

10	F10	20	100

5.4 EVALUATION OF NANOPARTICLES

5.4.1 DRUG ENTRAPMENT STUDY[67]

The efficiency study was determined by free drug content in the supernatant which is obtained after centrifuging the solid lipid suspension at (15,000rpm for 20 min at 0°C using ultra centrifuge) The absorbance was measured at 271 nm by UV spectrophotometrically .

5.4.2 INVITRO DRUG RELEASE STUDIES [68,33]

BY UV SPECTROPHOTOMETRIC METHOD

The invitro drug release study was carried out by using diffusion membrane technique. The nanoparticles preparation was placed in a dialysis membrane and it is dropped into a beaker containing 200 ml of diffusion medium (phosphate buffer saline 7.4) the medium was maintained at 37°C under magnetic stirring at constant speed .At fixed time interval 1ml of sample was taken from the diffusion medium for every 1 hour and it was replaced by 1ml fresh medium. This process was carried out for 24 hours. The sample was measured UV spectrophotometrically at 271nm. The

5.4.3 SCANNING ELECTRON MICROSCOPY (SEM)[70,24]

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM) .the sample for SEM analysis was mounted in the specimen using an adhesive small sample wad mounted directly in scotsch double adhesive tape. The sample was analyzed in hitachi scanning electron microscopy operated at 15Kv photograph was taken.

5.4.4 SURFACE CHARGE (ZETA POTENTIAL) DETERMINATION[71,45]

Zeta potential is an important parameter to evaluate and establish an optimum condition for stability of colloidal or dispersed systems. The prepared nanoparticle suspension were characterized with respect to zeta potential by using zeta potential analyser (Malvern Zeta Seizer).zeta potential is electrical charges on particles surface it create electrical barrier it is very important for drug stability. The effect of Eudragit RL 100 on the surface characterized of the nanoparticle was studied.

5.4.5 pH AND PHYSICAL APPEARANCE

The pH of the formulation was measured using pH meter. It plays a vital role in process of stability and formulation activity .The physical appearance of the formulation such as color and suspended foreign particulate matter were to be examined.

5.4.6 STABILITY STUDIES OF NANOPARTICLES[72]

The stability studies of nanoparticles involve observing the formulation at 45ºC/70% RH which accelerated condition and 4ºC on refrigerator and room temperature. The formulations were kept in both the temperature for 3 month and sufficient amount of sample were taken at perotic intervals for per formic the following tests,

a. Physical appearance
b. pH of the solution
c. In vitro drug release (Dissolution)
d. Percentage of drug entrapment

5.4.7 KINETICS OF DRUG RELEASE

The optimized formulation subjected to graphical treatment to assess the kinetic of drug release.

Zero order plot:

The optimized formulation is most suitable for parental administration as it founds to be good in the in vitro release kinetic study. The zero order plot obtained plot by plotting cumulative % drug release versus time

Higuchi plot:

The higuchi plot made by plotting cumulative %drug release versus square root of time.

Korsemeyer plot:

The graph obtained by log cumulative % drug release versus log time.

First order plot:

The graph obtained by log drug remaining versus log time.

6.0 RESULTS AND DISCUSSION

DEVELOPMENT OF FUROSEMIDE NANOPARTICLES:

In this study, Furosemide nanoparticles were prepared by solvent evaporation method by using Eudragit RL 100. Drug (Furosemide) and Polymer (Eudragit RL 100) (1:5) dissolved in ethanol. In other hand 0.50mg sodium dodecyl sulphate dissolved in 5ml water,then two solution were mixed. This mixture was homogenized by vortex for 1min and then probe sonication. Then this preparation evaporated by flash rotator evaporator for 20min.

Formulation with different ratios of polymer was prepared. Several physicochemical characteristics of nanoparticles such as morphology, particle size determination, drug release profile were investigated and stability of optimized formulation at various temperatures were evaluated.

DRUG & POLYMER COMPATABILITY STUDIES BY FTIR:

Pressed pellet technique was used to handle the sample in FTIR spectrometer. In this technique a pinch of sample was mixed with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted in to special holder of IR spectrometer.

IR spectrums for pure drug alone and physical mixture of drug and polymers are taken. The spectrum of physical mixture was compare with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture, hence there was to significant interaction between drug and excipients.

ENTRAPMENT EFFICIENCY OF NANOPARTICLE:

The Entrapment efficiency of Furosemide loaded nanoparticles were analyzed by dialysis method.

The formulation F1, F2, F3 polymer (Eudragir RL100) concentration 10mg, 20mg, 30mg is taken. The entrapment efficiency was 49%±0.14, 56%±0.11, 61% ±0.12 respectively. which shows less repulsive force between drug and polymer.

Table 13.Entrapment efficiency of nanoparticles

S.NO	Formulation code	Drug (mg)	Eudragit RL 100 (mg)	Entrapment efficiency (%)
1	F1	20	10	49±0.14
2	F2	20	20	56±0.11
3	F3	20	30	61±0.12
4	F4	20	40	67±0.09
5	F5	20	50	71±0.17
6	F6	20	60	77±0.12
7	F7	20	70	83±0.11
8	F8	20	80	88±0.08
9	F9	20	90	94±0.05
10	F10	20	100	45±0.04

Then further formulation F4, F5, F6 changes in polymer (Eudragit RL100) concentration as 40mg,50mg,60mg. The entrapment efficiency was 67%±0.12, 71% ±0.17, 77%±0.12 which also shows less repulsive force between drug and polymer.

So, further increase the concentration of polymer of formulation F7, F8, F9 polymer (Eudragit RL100) concentration as 70mg, 80mg, 90mg is taken. The entrapment efficiency was 83%±0.1, 88%±0.08, 94%±0.05. The formulation F9 gives high percentage efficiency 94%.which indicates the steady increase in entrapment efficiency.

Further study was carried out F10 changes in polymer concentration 100mg Eudragit RL100. Entrapment efficiency decreased to 45%±0.04 due to higher concentration of polymer. From the above result formulation F9 shows highest percentage of entrapment efficiency of 94%, so formulation F9 was selected for further studies.

IN VITRO DRUG RELEASE PROFILE OF NANOPARTICLES

- The in vitro drug release of Furosemide nanoparticle carried out by membrane diffusion method and in vitro drug release study was carried out for 24 hours .
- The in vitro drug release of Furosemide loaded nanoparticles with Eudragit RL 100 polymer.
- The in vitro drug release of formulation F1(Furosemide 20mg,Eudragit RL100 10mg) . The percentage of drug release was 98.43% in 6 hours. Formulation releases the drug in 6 hours.
- So, further formulation F2, F3 with different concentration of polymer (Furosemide 20mg with Eudragit RL 100 20mg, 30mg) the percentage of drug release was respectively 97.44%, 96.43% in 8 hours. Which formulations were shows quick release (8hours). Due to very low concentration of polymer.
- The formulation F4, F5, F6, F7 carried out with increase the polymer concentration the percentage of drug release was, 98.46 in 13 hours, 98.46% in 15 hours, 96.45% in 19hours, 98.47% in 20 hours, due to less repulsive force.
- The formulation F8, F9 with increase the concentration of polymer concentration the percentage of drug release was 92.43 % in 24 hours, 98.47 in hours.
- The formulation F10 (Furosemide 20 with Eudragit RL 100 200mg)the percentage of drug release was 55.27% in 24 hours . the drug release is 55.27% due to increase the polymer concentration.
- From the above all formulations (F1-F10) It confirms that the percentage of drug release was satisfactory in formulation F9 (20mg of Furosemide with 180mg of Eudragit RL 100) Drug release was 98.47% ,so it was decided to be optimized formulation for further study.

Table 14. In vitro drug release for F1

S.NO	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	2.2	2.3	23
2	3.8	3.81	38.10
3	5.5	5.51	55.19
4	7.3	7.37	73.27
5	8.6	8.63	86.35
6	9.8	9.84	98.43

Fig No:2 IN VITRO DRUG RELEASE FOR FORMULATION F1

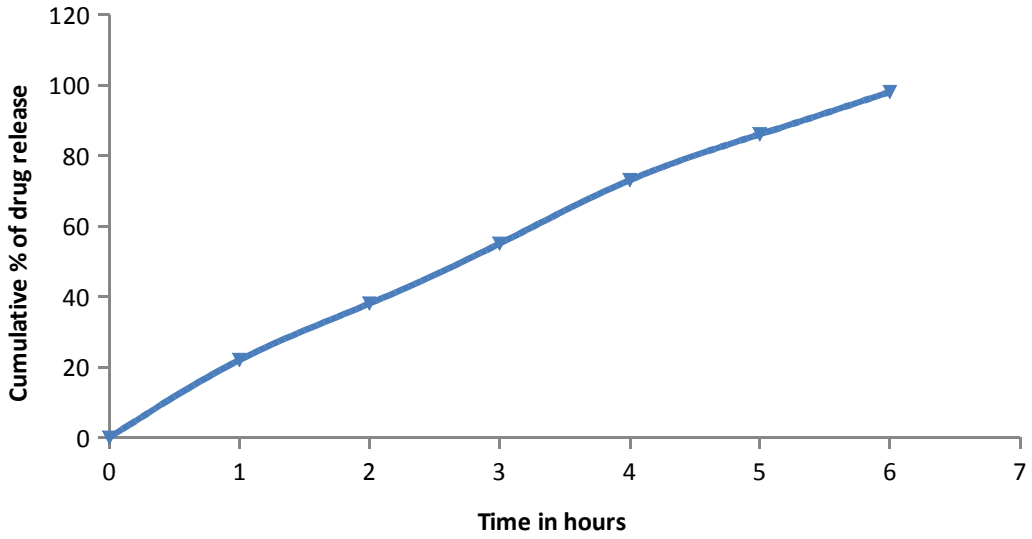


Table 15. In vitro drug release for F2

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	2.0	2.0	20
2	3.2	3.22	32.2
3	4.5	4.51	43.16
4	5.4	5.42	54.22
5	6.9	6.92	66.27
6	8.0	8.03	78.30
7	8.9	8.94	89.40
8	9.7	9.74	97.44

Fig No : 3 IN VITRO DRUG RELEASE FOR FORMULATION F2

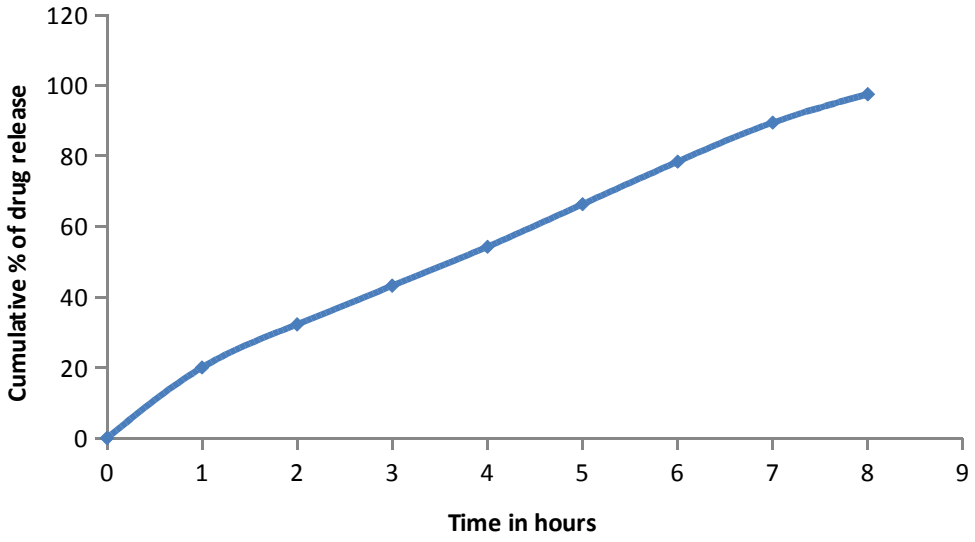


Table 16. In vitro drug release for F3

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	1.8	1.8	18
2	2.9	2.90	29.09
3	4.1	4.14	41.14
4	5.2	5.22	52.20
5	6.7	6.72	67.26
6	7.7	7.73	77.33
7	8.6	8.63	86.38
8	9.6	9.64	96.43

Fig No: 4 IN VITRO DRUG RELEASE FOR FORMULATION F3

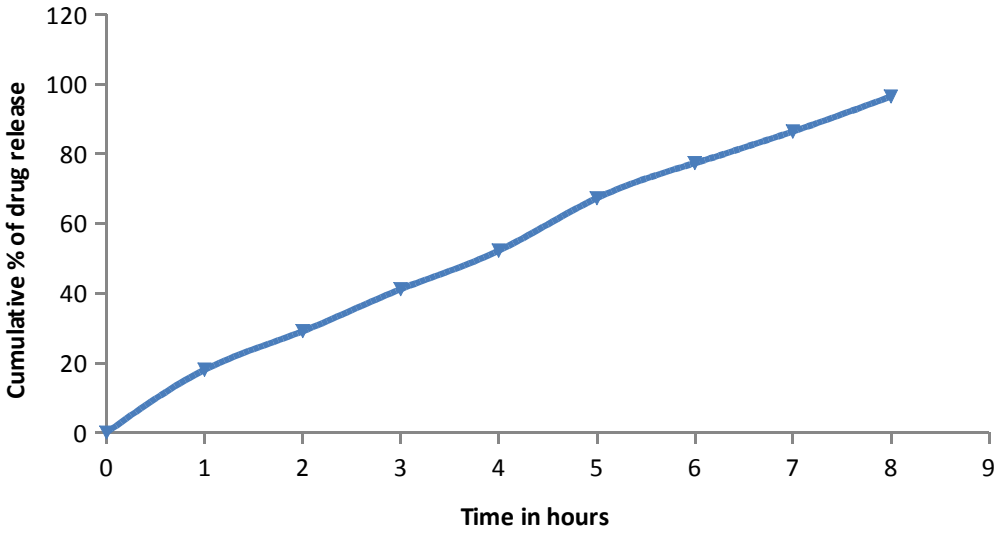


Table 17. IN VITRO DRUG RELEASE FOR F4

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	1.0	1.0	10
2	1.9	1.90	19.05
3	2.6	2.60	26.09
4	3.5	3.51	35.13
5	4.3	4.31	43.17
6	5.6	5.62	56.21
7	6.3	6.32	63.28
8	7.0	7.03	70.31
9	7.7	7.73	77.35
10	8.3	8.33	83.38
11	8.8	8.84	88.41
12	9.2	9.24	92.44
13	9.8	9.84	98.46

Fig No : 5 IN VITRO DRUG RELEASE FOR F4

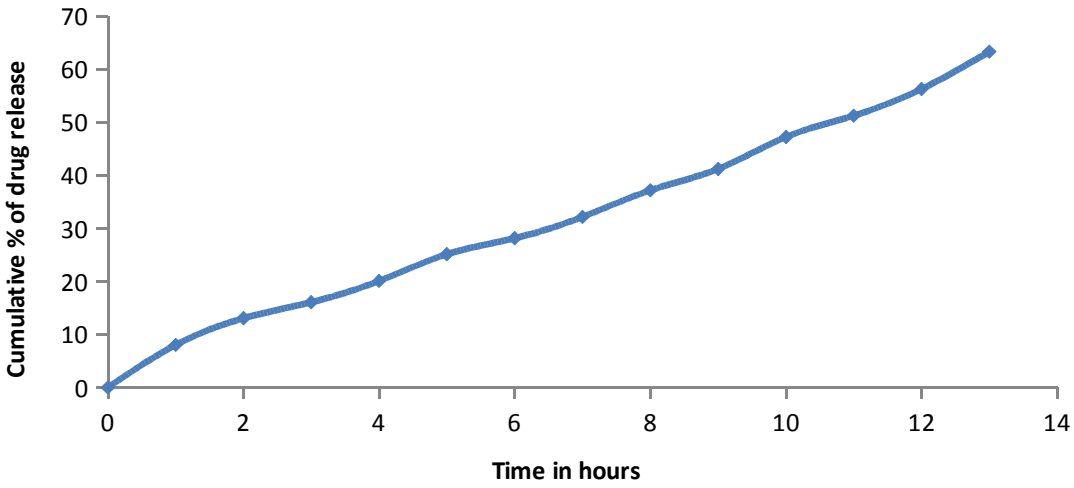


Table 18. IN VITRO DRUG RELEASE FOR F5

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	1.0	1.0	10
2	1.7	1.70	17.05
3	2.3	2.30	23.08
4	3.1	3.11	31.11
5	3.8	3.81	38.15
6	4.7	4.71	47.19
7	5.3	5.32	53.23
8	6.1	6.12	61.26
9	6.7	6.73	67.30
10	7.1	7.13	71.33
11	7.8	7.83	78.33
12	8.3	8.33	83.39
13	8.7	8.74	87.41
14	9.3	9.34	93.43
15	9.8	9.84	98.46

Fig No :6 IN VITRO DRUG RELEASE FOR FORMULATION F5

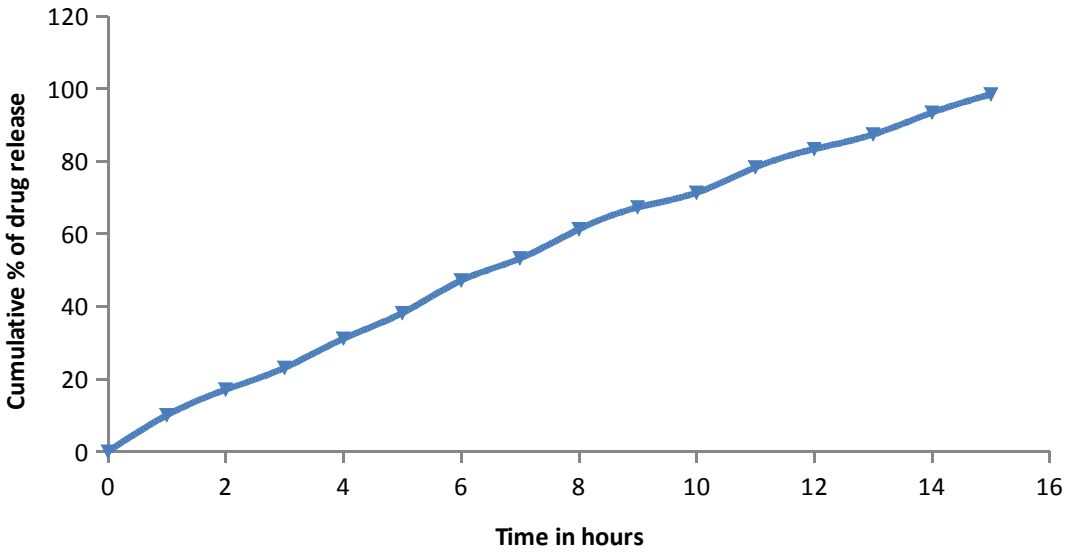


Table 19. IN VITRO DRUG RELEASE FOR F6

Time (Hrs)	Amount of drug release (mg)	Cumulative amuont of drug release	Cumulative % drug release
1	0.8	0.8	8
2	1.3	1.30	13.04
3	1.6	1.60	16.06
4	2.0	2.00	20.08
5	2.5	2.51	25.10
6	2.8	2.81	28.12
7	3.2	3.21	32.14
8	3.7	3.71	37.16
9	4.1	4.11	41.18
10	4.7	4.72	47.20
11	5.1	5.12	51.23
12	5.6	5.62	56.25
13	6.3	6.32	63.28
14	6.8	6.83	68.31
15	7.6	7.63	76.31
16	8.2	8.23	82.38
17	8.6	8.64	86.41
18	9.1	9.14	91.43
19	9.6	9.64	96.45

Fig No : 7 IN VITRO DRUG FOR FORMULATION F6

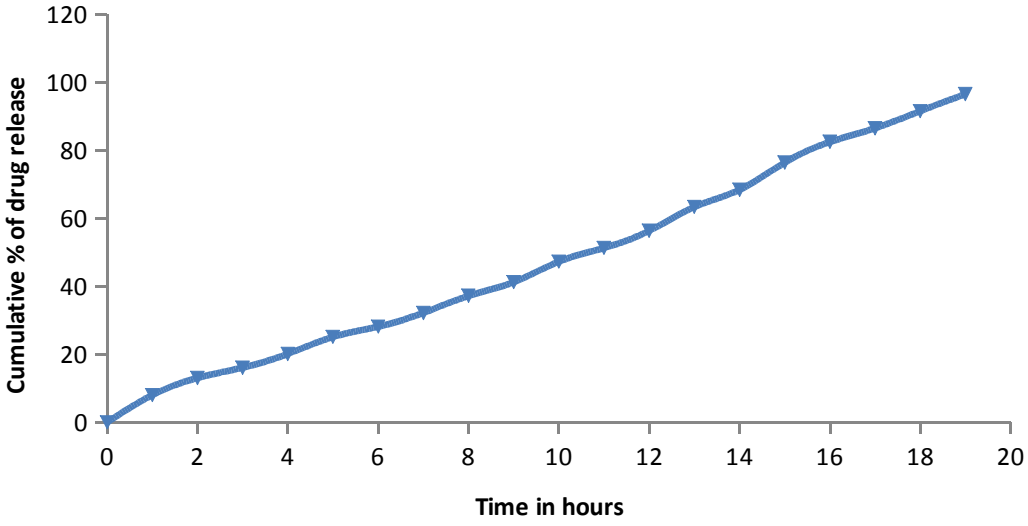


Table 20. IN VITRO DRUG RELEASE FOR F7

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	0.6	0.6	6
2	1.1	1.10	11.03
3	1.5	1.50	15.05
4	1.8	1.80	18.07
5	2.2	2.20	22.09
6	2.7	2.71	27.11
7	3.2	3.21	32.13
8	3.7	3.71	37.16
9	4.2	4.21	42.18
10	4.7	4.72	47.21
11	5.3	5.32	53.23
12	5.7	5.72	57.26
13	6.1	6.12	61.28
14	6.6	6.63	66.30
15	7.1	7.13	71.33
16	7.7	7.73	77.35
17	8.3	8.33	83.38
18	8.8	8.84	88.41
19	9.3	9.34	93.44
20	9.8	9.84	98.47

Fig No : 8 IN VITRO DRUG RELEASE FOR FORMULATION F7

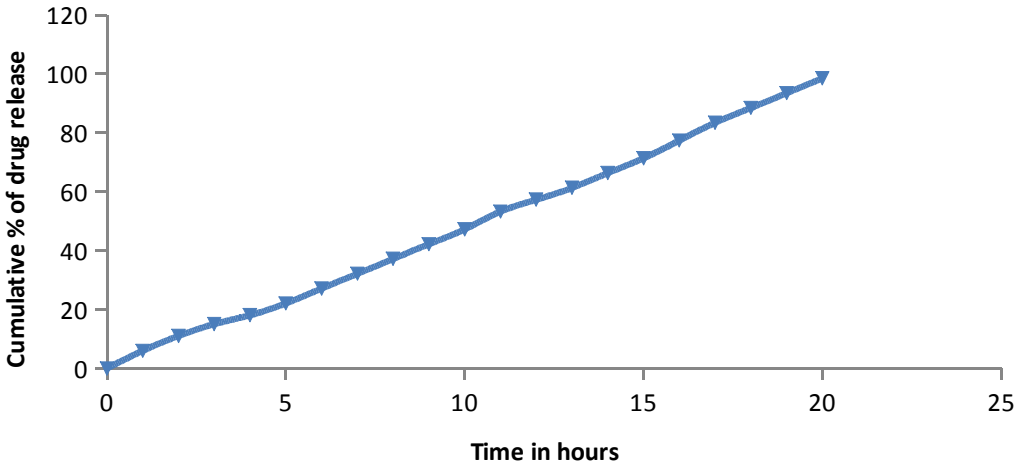


Table 21. VITRO DRUG RELEASE FOR F8

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release	Cumulative % drug release
1	0.4	0.4	4
2	0.6	0.60	6.02
3	0.9	0.90	9.03
4	1.3	1.30	13.04
5	1.8	1.80	18.06
6	2.3	2.30	23.09
7	2.8	2.81	28.11
8	3.3	3.31	33.14
9	3.8	3.81	38.16
10	4.2	4.21	42.19
11	4.5	4.52	45.22
12	4.9	4.92	49.22
13	5.1	5.12	51.24
14	5.3	5.32	53.25
15	5.8	5.82	58.26
16	6.3	6.32	63.29
17	6.8	6.83	68.31
18	7.1	7.13	71.34
19	7.3	7.33	73.35
20	7.6	7.63	76.36
21	8.0	8.03	80.38
22	8.3	8.34	83.40
23	8.7	8.74	87.41
24	9.2	9.24	92.43

Fig No : 9 IN VITRO DRUG RELEASE FOR FORMULATION F8

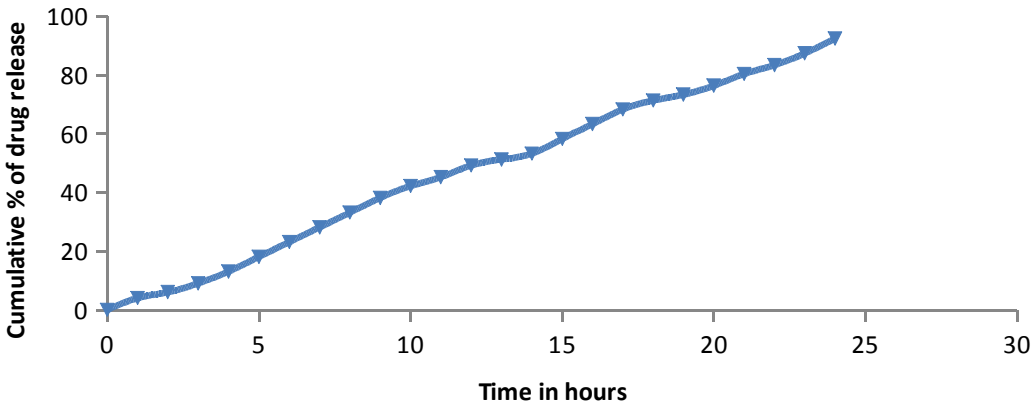


Table 22. I N VITRO DRUG RELEASE FOR F9

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	03.	0.3	3
2	0.7	0.70	7.01
3	1.2	1.20	12.03
4	1.6	1.60	16.06
5	2.1	2.10	21.08
6	2.7	2.71	27.10
7	3.2	3.21	32.13
8	3.8	3.81	38.16
9	4.3	4.31	43.19
10	4.6	4.62	46.21
11	5.0	5.02	50.23
12	5.4	5.42	54.25
13	5.8	5.82	58.27
14	6.1	6.12	61.29
15	6.5	6.53	65.30
16	6.9	6.93	69.32
17	7.3	7.33	73.34
18	7.8	7.83	78.36
19	8.1	8.13	81.39
20	8.5	8.54	85.40
21	8.8	8.84	88.42
22	9.1	9.14	91.44
23	9.4	9.44	94.45
24	9.8	9.84	98.47

Fig No : 10 IN VITRO DRUG RELEASE FOR FORMULATION F9

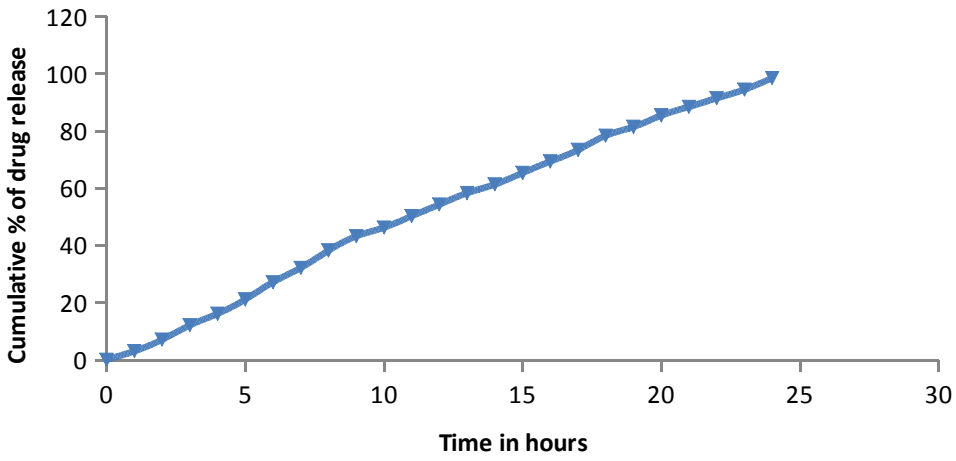
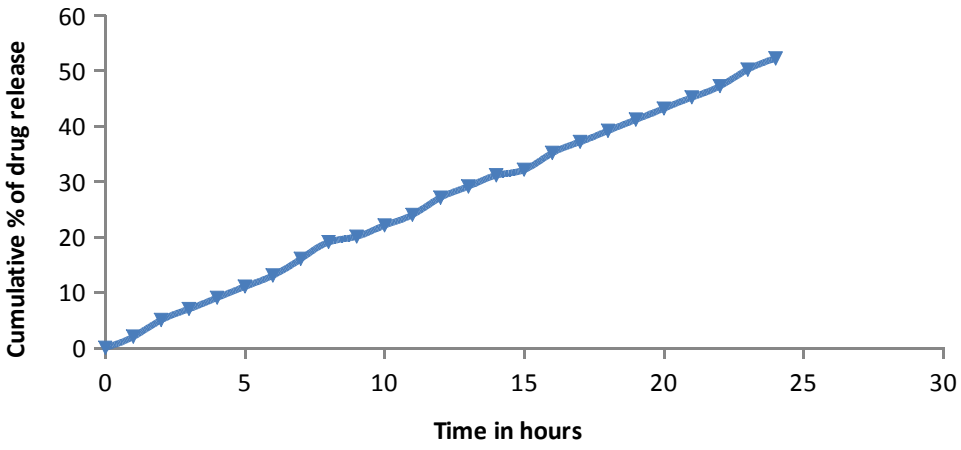


Table 23. IN VITRO DRUG RELEASE FOR FORMULATION F10

Time (Hrs)	Amount of drug release (mg)	% of drug release	Cumulative % drug release
1	0.2	0.2	2
2	0.5	0.50	5.01
3	0.7	0.70	7.02
4	0.9	0.90	9.03
5	1.1	1.10	11.04
6	1.3	1.30	13.05
7	1.6	1.60	16.06
8	1.9	1.90	19.08
9	2.0	2.00	20.09
10	2.2	2.21	22.1
11	2.4	2.40	24.01
12	2.7	2.71	27.12
13	2.9	2.91	29.13
14	3.1	3.11	31.14
15	3.2	3.21	32.15
16	3.7	3.71	37.17
17	3.9	3.91	39.18
18	4.1	4.11	41.19
19	4.3	4.32	43.20
20	4.5	4.51	45.12
21	4.7	4.72	47.22
22	5.0	5.02	50.23
23	5.2	52.2	52.25
24	5.5	5.52	55.27



SCANNING ELECTRON MICROSCOPY

The surface characteristics of optimal formulation (F9) particle size were studied by scanning electron microscopy. SEM image of prepared nanoparticle formulation shows the coating of polymer mixture on drug particle. The appearance of nanoparticles in scanning electron microscopy in granules form, which indicates a thin and uniform coating over the drug.

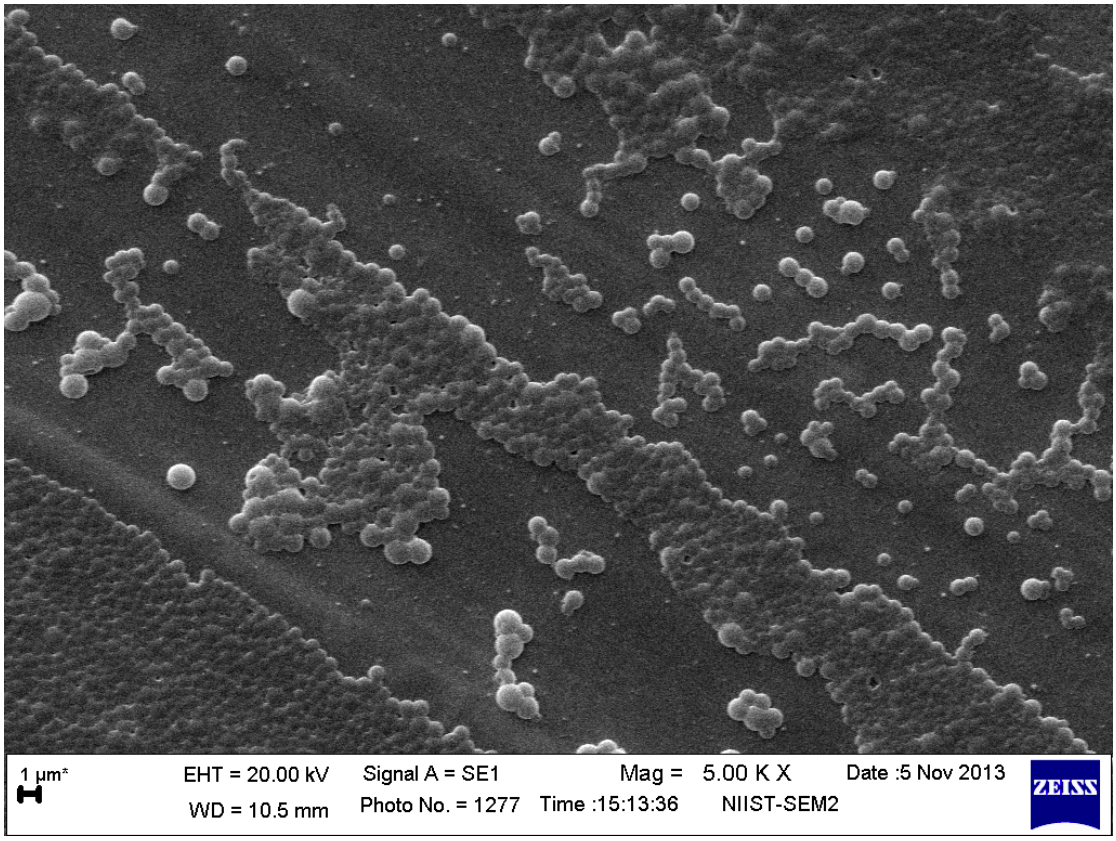


Fig No : 12 SEM FOR F9

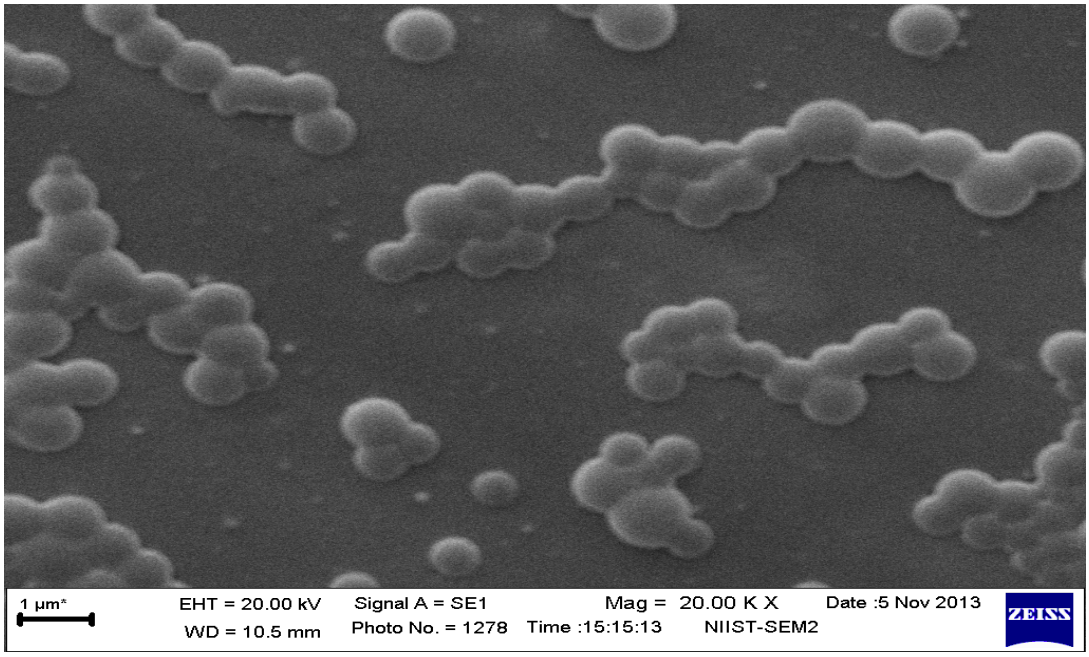


Fig No : 13 SEM FOR F9

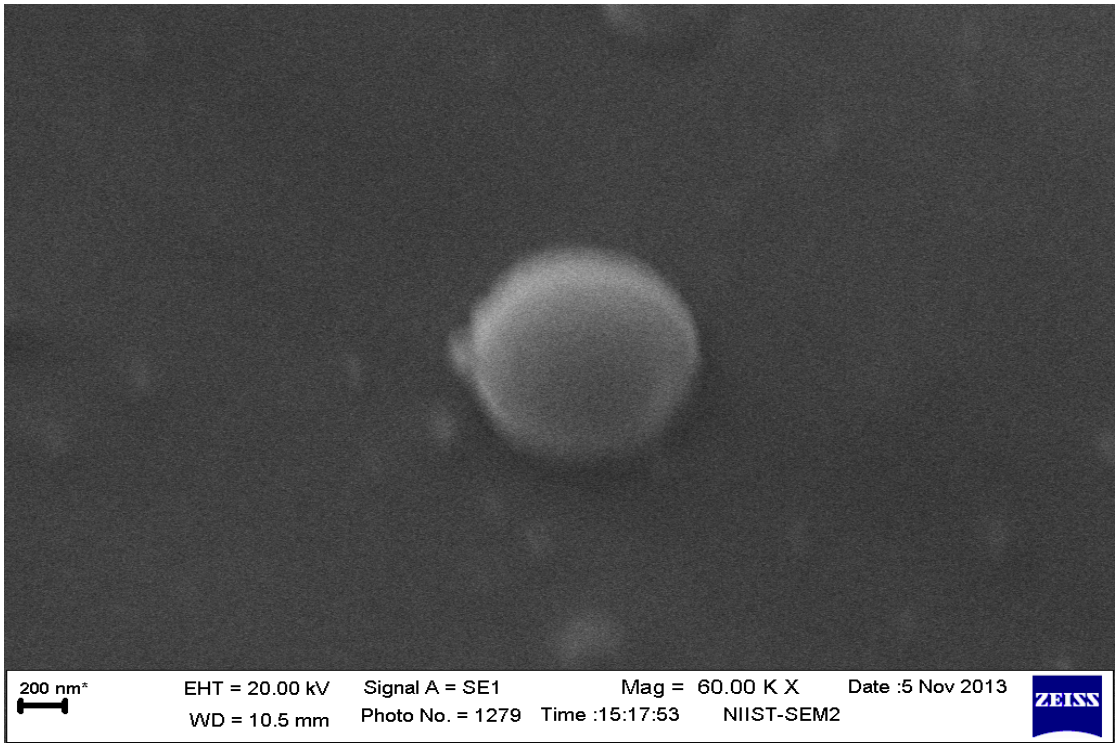


Fig No : 14 SEM FOR F9

SURFACE CHARGE (ZETA POTENTIAL)

The potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticle. It reflects the electrical potential of particles is influenced by the composition of the particles and which it is dispersed. When nanoparticle formulation are administrated through intravenous route they are easily identified and detected by the phagocytes. The particle size and the hydrophobicity surface of the nanoparticle determine the adsorption of blood components (proteins) called as opsonins. These opsonins in turn decide the fate of the nanoparticles. Binding of these opsonins on to the surface is known as opsonization. Non modified nanoparticles were rapidly opsonized and gets easily eliminated from the body. Hence,to increase the likehood of the success in drugtargeting by nanoparticles,it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo .

The zeta potential of the nanoparticle formulation with Eudragit RL 100 which present in the formulation are de-aggregated and remain same and more stable in the suspension and zeta potential (mV) is 59.0 and zeta deviation (Mv) is 5.29 and conductivity (Ms/CM) is 0.086. So polymer is more suitable for nanoparticle preparation and the result shows smooth surface character repelled action and it decrease the opsonization.

Zeta Potential Report
V2.2
Malvern Instruments Ltd - @ Copyright 2008

Sample Details

Sample Name: Sample - 1 (Furosemide Nano Particles) 2
SOP Name : mansettings.nano
General Notes :

File Name :KMCP.dts
Record Number:8
Date and Time: Friday, 13 Dec 2013

Dispersant Name : Water
Dispersant RI : 1.330
Viscosity (mpa...) : 0.8872
Dispersant Dielectric Constant: 78.5

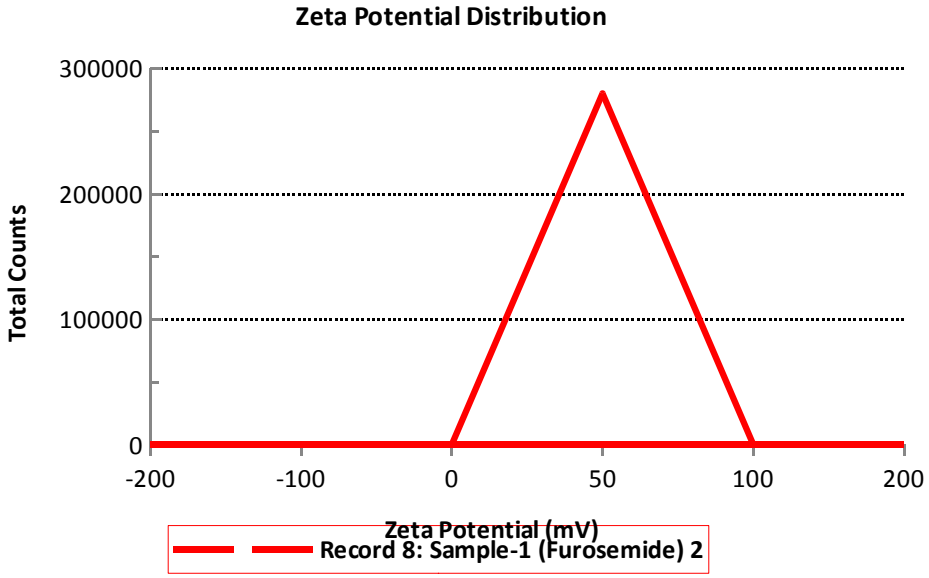
System

Temperature (C) :25.0
Count Rate (kcps) :235.2
Cell Description : Clear disposable zeta cell

Zeta Runs : 12
Measurement Position(mm): 2.00
Attenuator: 6

Results

	Mean (mV)	Area (%)	Width (mV)		
Zeta Potential (mV) :	59.0	**Peak 1:** 59.0	100.0	5.29	
Zeta Deviation (mV) :	5.29	**Peak 2:** 0.00	0.0	0.00	
Conductivity (mS/cm):	0.0866	**Peak 3:** 0.00	0.0	0.00	
Result Quality :	**Good**				



Zetasizer Ver.6.20
Serial Number : MAL1045544

File Name : KMCP.dts
Record Number : 8
13 Dec 2013 10.45:52 AM

STABILITY STUDIES OF FUROSEMIDE NANOPARTICLES:

The stability studies of optimized nanoparticle formulation F10 was carried our for 3 months .The test was performed in three conditions 4° C, Room temperature and 45°C/70% RH. At the time interval of one month the nanoparticle formulation were evaluated for entrapment efficiency. The stability of nanoparticles formulation was more stable in refrigerator (4°C) when compared to room temperature and at (45°C/70%RH)

Table 24. Stability studies for Furosemide nanoparticle

S.NO	Storage Condition	Test parameters	1st month	2th month	3rd month
1	4°C	pH	7.4	7.4	7.4
		colour	Clear& colour less	Clear& colour less	Clear & colour less
		Cumulative % drug release	98.47	97.25	96.88
2	Room Temperature	pH	7.4	7.4	7.3
		colour	Clear & colour less	Clear & colour less	Clear & colour less
		Cumulative % drug release	98.47	93.36	91.85
3	Acceleration condition at 45°C/70°% RH		7.4	7.3	7.3
			clear& colour less	Clear& colour less	Clear&colour less
			95.10	91.21	89.24

Table 25. In vitro release for optimized formulation F10 stability study at 4ºC

Time (Hrs)	Cumulative % drug release		
	1st month(%)	2nd month	3rd month
1	3	3	2.8
2	7.01	7.01	6.98
3	12.03	12.00	11.96
4	16.06	16.02	15.98
5	21.08	21.04	21.00
6	27.10	27.04	27.00
7	32.13	32.08	31.98
8	38.16	38.02	37.96
9	43.19	43.06	42.92
10	46.21	45.96	45.88
11	50.23	49.90	49.76
12	54.25	53.91	53.63
13	58.27	57.54	5.22
14	61.29	60.88	60.14
15	65.30	64.50	63.96
16	69.32	69.02	68.86
17	73.34	72.14	72.02
18	78.36	78.03	77.95
19	81.39	80.68	80.69
20	85.40	84.66	84.22
21	88.42	87.21	87.01
22	91.44	91.17	91.15
23	94.45	94.02	93.57
24	98.47	97.25	96.88

STABILITY STUDY RELEASE DATA FOR FORMULATION F10 AFTER 3 MONTHS AT 4ºC

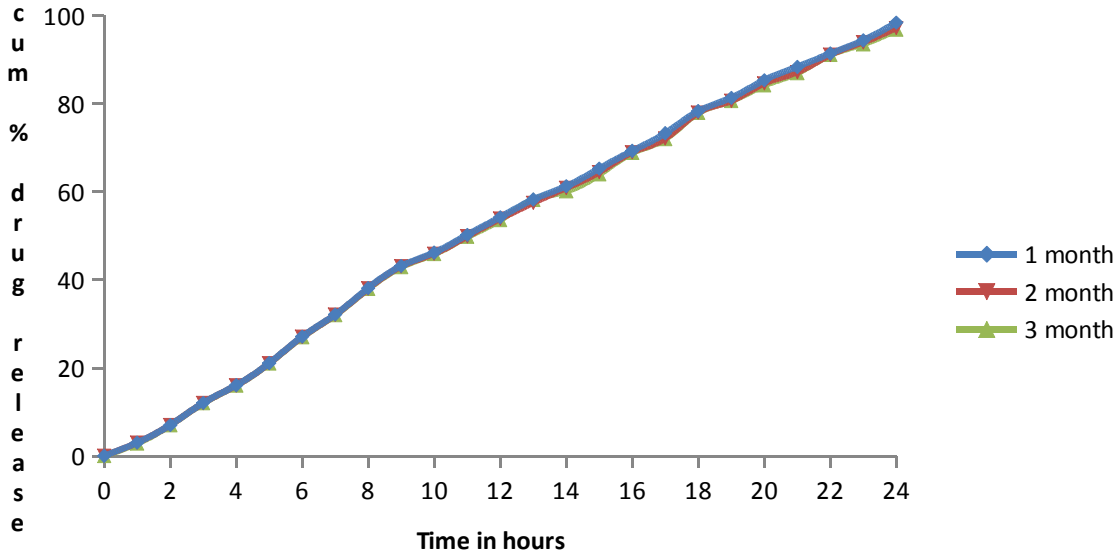

cum % drug release
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours
1 month
2 month
3 month

Table 26. IN VITRO DATA FOR OPTIMIZED FORMULATION F10 STUDY AT ROOM TEMPERATURE

Cumulative % drug release			
Time (Hrs)	**1st month(%)**	**2nd month**	**3rd month**
1	3	2.8	2.7
2	7.01	6.88	5.12
3	12.03	9.92	8.21
4	16.06	14.98	12.10
5	21.08	19.12	16.14
6	27.10	23.16	21.18
7	32.13	28.20	25.52
8	38.16	34.28	32.58
9	43.19	40.32	37.66
10	46.21	44.42	41.72
11	50.23	48.50	43.78
12	54.25	51.56	47.87
13	58.27	54.63	50.95
14	61.29	57.69	54.02
15	65.30	62.75	59.08
16	69.32	66.55	63.16
17	73.34	71.62	68.22
18	78.36	75.68	72.32
19	81.39	78.76	76.38
20	85.40	80.85	78.48
21	88.42	84.96	81.56
22	91.44	87.06	85.66
23	94.45	90.18	88.72
24	98.47	93.36	91.85

STABILITY STUDY RELEASE DATA FOR FORMULATION F10 AFTER THREE MONTHS AT ROOM TEMPERATURE

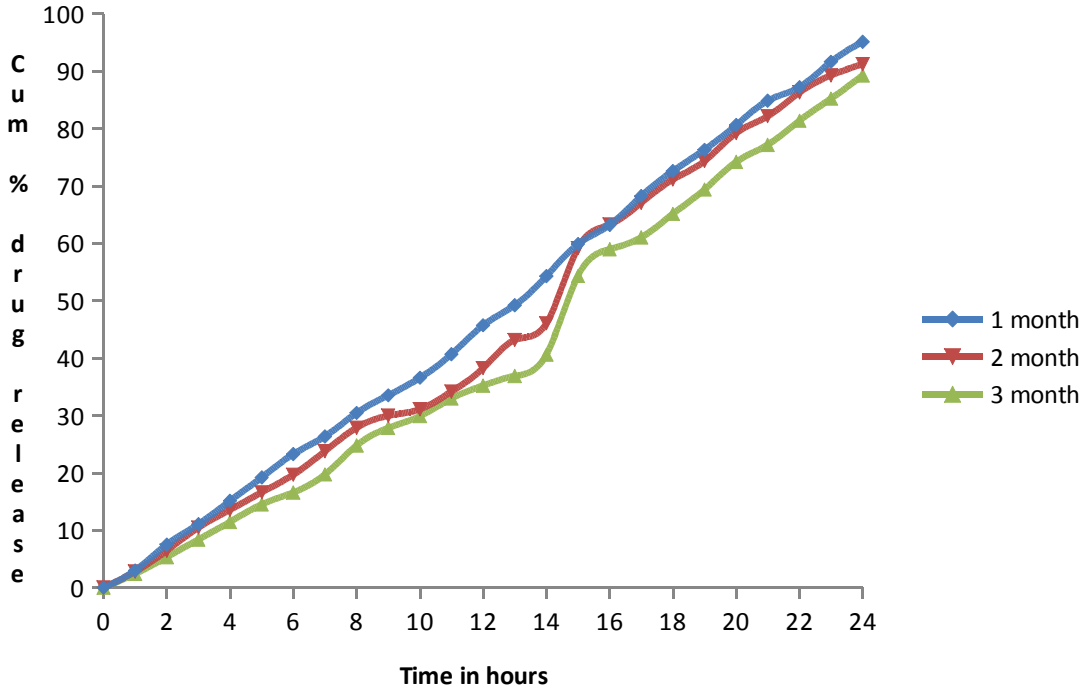
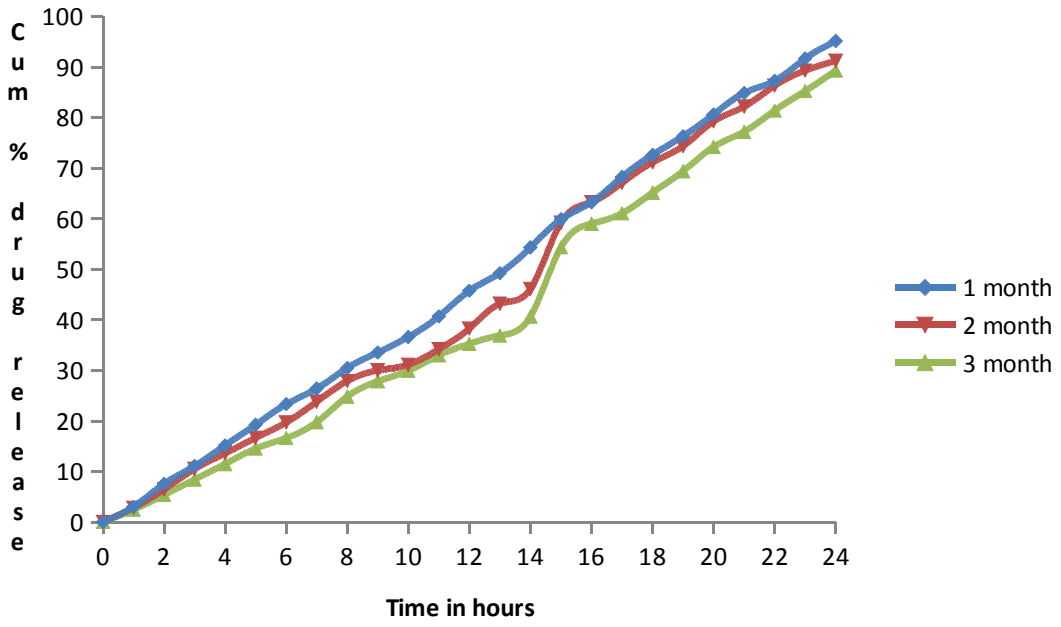


Table 27. IN VITRO DATA FOR OPTIMIZED FORMULATION F10 STUDY AT 45^{O}C/75% RH

Cumulative % drug release			
Time (Hrs)	**1st month(%)**	**2nd month**	**3rd month**
1	3	2.8	2.4
2	7.52	6.44	5.32
3	11.06	10.48	8.39
4	15.14	13.56	11.45
5	19.20	16.62	14.51
6	23.28	19.68	16.58
7	26.36	23.78	19.72
8	30.44	27.86	24.78
9	33.50	29.96	27.83
10	36.56	31.08	29.89
11	40.66	34.16	32.96
12	45.72	38.25	35.21
13	49.21	43.15	36.87
14	54.28	46.06	40.61
15	59.85	59.16	54.31
16	63.21	63.26	58.98
17	68.24	67.06	61.03
18	72.59	71.12	65.13
19	76.29	74.32	69.36
20	80.58	79.21	74.17
21	84.84	82.13	77.16
22	87.21	86.28	81.36
23	91.62	89.26	85.21
24	95.10	91.21	89.24

IN VITRO DATA FOR OPTIMIZED FORMULATION F10 STUDY AT 45°/75% RH

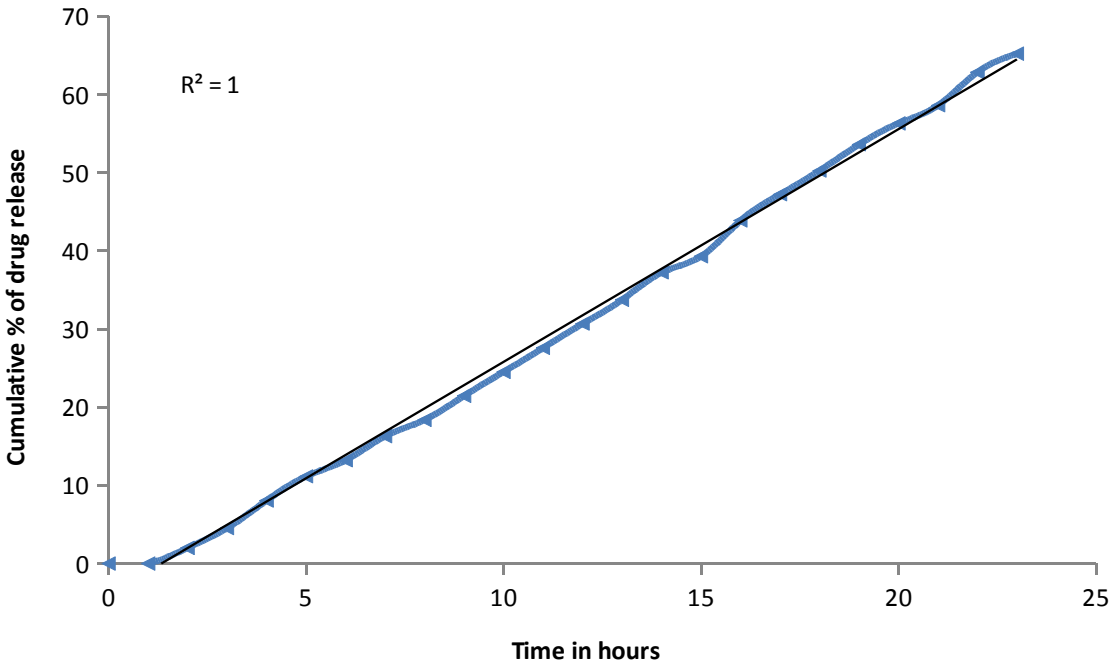


Stability Discussion:

The Stability tests were carried out for a period of 3 months at various conditions. The results showed that the formulation remains stable through out the period of study.

Kinetics of drug release for optimized formulation F10

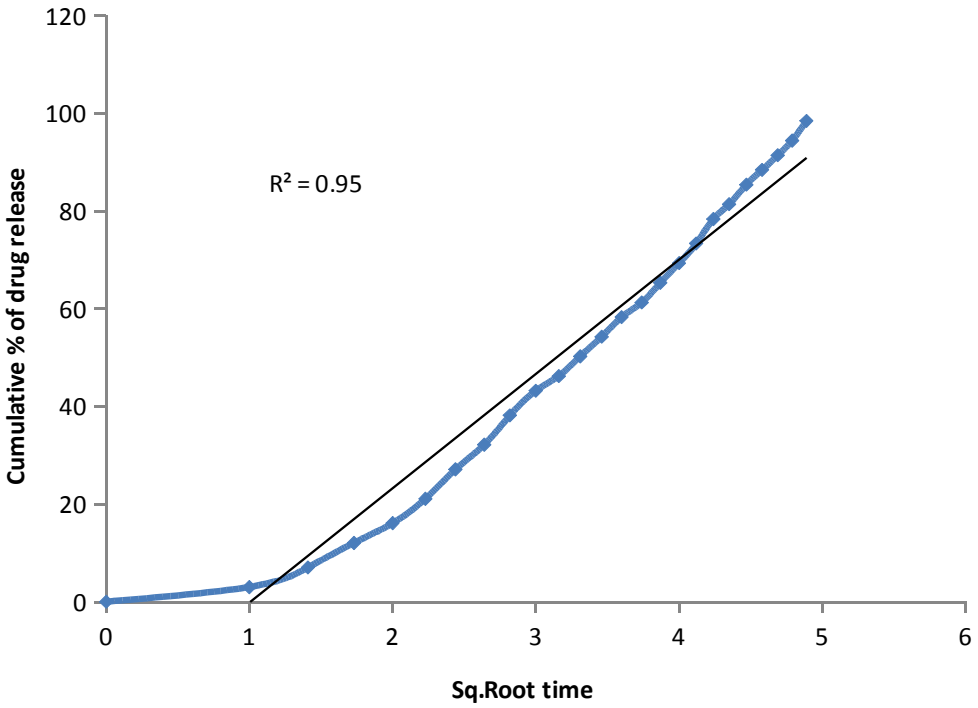
The optimized formulation F9 was introduced into graphical treatment for kinetics of drug release



Regression=0.998

The optimized formulation F10 of nanoparticle is more suitable for parentral administration it shows good in the in vitro release kinetic study. The zero order plots were obtained by plotting cumulative percentage drug release. The regression value is 0.998. confirm that it follow zero order release.

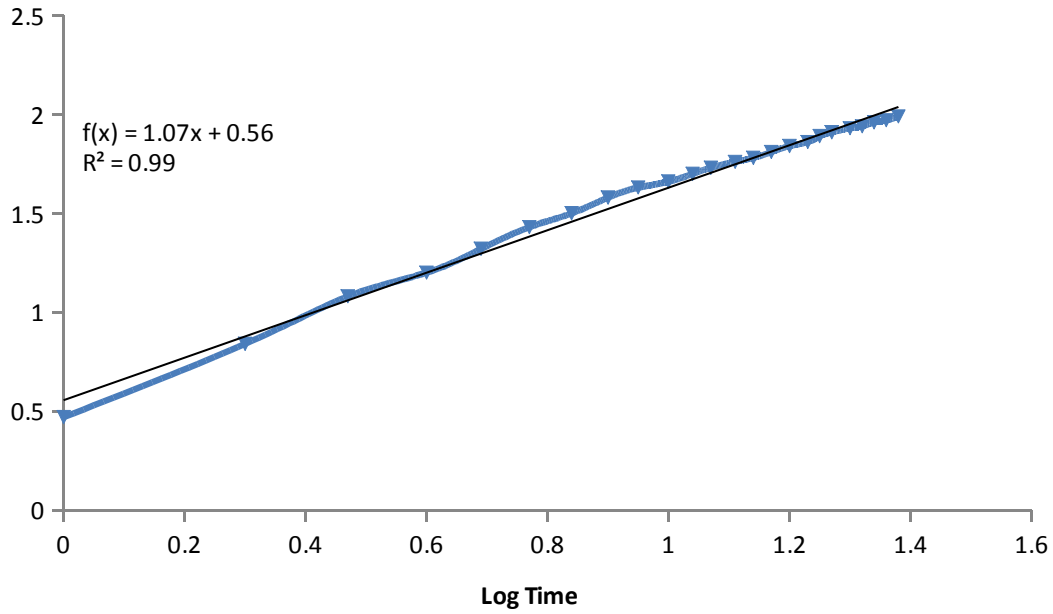
HIGUCHI'S PLOT :



Regression = 0.954

Higuchi plot was made by plotting cumulative % of drug release against square root of time. The regression value was found to be 0.954 .This indicates that diffusion is one of the mechanism of drug release.

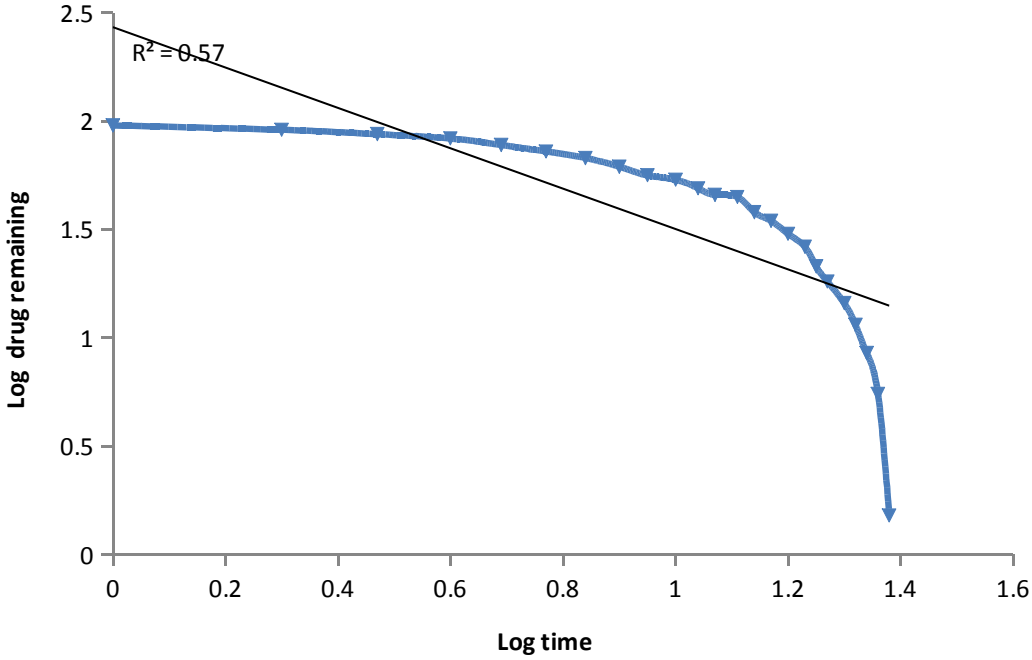
KORSEMEYER PLOT:



n=1.073

The graph was plotted between log cumulative % of drug release and log time. The value was found to be $0.45 < n < 0.89$ anomalous (non-fickian) diffusion. This indicates that the diffusion non-fickian for the mechanism of drug release.

FIRST ORDER RELEASE:



R=0.571

R value indicates that drug release not follow first order kinetics.

7.0 SUMMARY AND CONCLUSION

The present study Furosemide nanoparticle developed a nanoparticulate drug delivery system of using biodegradable polymer Eudragit RL100.

All batches of nanoparticles (F1-F10) were prepared by solvent evaporation method.

The entrapment efficiency of the optimized formulation was 94±0.04, and invitro drug release was 98.16 after 24 hours. It also obeys the zero order, follows. Particle size determination by Scanning electron microscope shows the best formulation containing size of about 200nm. The stability test performed revealed that the formulation was good. The best formulation was examined for zeta potential determination.

The formulation F9 showed maximum deviation of -59mV which demonstration that the particles are separate and highly repelling. This repelling property found to be more useful in decreasing opsonization by membrane filtration and favours specificity.

The Stability tests were carried out for a period of 3 months at various conditions. The results showed that the formulation remains stable through out the period of study.

The optimized formulation F9 was found to follow zero order release pattern. Which was revealed by linearity shown from the plot of time versus concentration.

In future F9 can be subjected to bio-equivalence study and suitability to market can be decided on that.

Thus aim of the project was achieved by optimizing the formulation parameter.

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